


12-1-2011

Multiple Storm Event Impacts on Epikarst Storage and Transport of Organic Soil Amendments in South-Central Kentucky

Sean M. Vanderhoff

Western Kentucky University, sean.vanderhoff618@topper.wku.edu

Follow this and additional works at: <http://digitalcommons.wku.edu/theses>

 Part of the [Environmental Indicators and Impact Assessment Commons](#), [Hydrology Commons](#), [Physical and Environmental Geography Commons](#), and the [Water Resource Management Commons](#)

Recommended Citation

Vanderhoff, Sean M., "Multiple Storm Event Impacts on Epikarst Storage and Transport of Organic Soil Amendments in South-Central Kentucky" (2011). *Masters Theses & Specialist Projects*. Paper 1128.
<http://digitalcommons.wku.edu/theses/1128>

This Thesis is brought to you for free and open access by TopSCHOLAR®. It has been accepted for inclusion in Masters Theses & Specialist Projects by an authorized administrator of TopSCHOLAR®. For more information, please contact connie.foster@wku.edu.

MULTIPLE STORM EVENT IMPACTS ON EPIKARST STORAGE AND
TRANSPORT OF ORGANIC SOIL AMENDMENTS IN SOUTH-CENTRAL
KENTUCKY

A Thesis
Presented to
The Faculty of the Department of Geography and Geology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Sean Vanderhoff

December 2011

MULTIPLE STORM EVENT IMPACTS ON EPIKARST STORAGE AND
TRANSPORT OF ORGANIC SOIL AMENDMENTS IN SOUTH-CENTRAL
KENTUCKY

Date Recommended _____



Jason Polk, Director of Thesis




Chris Groves



Leslie North



Carl Bolster



Dean, Graduate Studies and Research 18-JAN-2012
Date

ACKNOWLEDGMENTS

Throughout this research numerous individuals have contributed their time and efforts to this project. First and foremost, I would like to thank Carter. Without all of your constant love and support, I may not have seen it to the end. You truly are the driving force behind all that I do. I would like to thank my family for all of their continuous support, even if you didn't understand what I was studying or why I wanted to pursue this research, you stood behind me 100 percent.

Many thanks go out to my colleagues at the Hoffman Institute. Your assistance in fieldwork and data compilation did not go unnoticed or unappreciated. This could not have been done without you. I would like to thank Jonathan Oglesby and the WATERS laboratory for their lab work and analysis. Many thanks go to Lee Anne Bledsoe and Celia Davis at the Crawford Hydrology Lab. Your insight to interpretation of the data is tremendously appreciated.

There are not enough 'thank yous' in the world to truly say how much Ben Miller has meant to me in this process. I truly look to you as a mentor and a good friend.

I would like to thank committee member Dr. Carl Bolster at the Agriculture Research Service in Bowling Green, KY for guidance and the United States Department of Agriculture for funding and technical support.

I would like to thank my committee members at Western Kentucky University Dr. Leslie North, Dr. Chris Groves and Dr. Jason Polk. Through each of your individual talents you have helped me turn an idea into a reality.

Dr. North has very much helped in my growth as a writer and my thinking of how the natural world can shape our policies. Dr. Groves has been an incredible factor in my development. Your continued support not only in karst research, but also in life, will never be forgotten. This thesis would not have been completed had it not been for Dr. Polk. I am truly thankful to have an advisor who was as dedicated to one student as Dr. Polk was. I am truly a better person for having you in my life.

TABLE OF CONTENTS

LIST OF FIGURES.....	vii
ABSTRACT.....	ix
CHAPTER ONE: INTRODUCTION	1
1.1 Examination of Epikarst.....	4
1.2 Fecal Bacteria Survivability in Soil and Water.....	6
1.3 Best Management Practices.....	8
CHAPTER TWO: STUDY AREA.....	10
CHAPTER THREE: METHODOLOGY.....	15
3.1 Fluorescent Dye Tracing	19
3.2 Analytical Methodology.....	21
CHAPTER FOUR: RESULTS.....	23
4.1 Dye Traces.....	23
4.2 Seasonal Results.....	30
CHAPTER FIVE: DISCUSSION.....	33
5.1 Dye Traces.....	34
5.2 Fecal Coliform Indication	37

5.3 Seasonal Storm Events.....	39
5.4 Possible Scenarios of Hydrological Characteristics of Soil - Epikarst Movement.....	41
5.5 Best Management Practices Recommendations.....	43
5.6 Importance of Seasonal Storm Event Sampling	45
5.7 Conclusions.....	46
5.8 Future Studies	47
APPENDIX A Sulphorhodamine B dye trace.....	48
APPENDIX B Fluorescein dye trace.....	49
APPENDIX C Eosine dye trace.....	51
APPENDIX D <i>E. coli</i> and fecal coliform data.....	55
APPENDIX E Discharge barrel calculation.....	60
REFERENCES.....	62

LIST OF FIGURES

Figure 1.1 Epikarst Model.....	5
Figure 2.1 Plan View Map of Crumps Cave.....	11
Figure 2.2 Overlay of Crumps Cave and surrounding area.....	14
Figure 3.1 Waterfall One and set up of discharge barrel and dataloggers.....	17
Figure 3.2 Dye Injection of eosine on April 25 th 2011.....	21
Figure 4.1 SpC data at WF1 January 30 th - February 7 th	24
Figure 4.2 FC counts a WF1 January 30 th - February 7 th	25
Figure 4.3 sulphorhodamine B at WF1 February 1 st - March 1 st	26
Figure 4.4 Fluorescein at WF1 February 23 rd - March 9 th	27
Figure 4.5 Eosine at WF1 April 25 th - May 10 th	28
Figure 4.6 SpC at WF1 April 25 th - May 10 th	29
Figure 4.7 FC counts at WF1 April 25 th - May 10 th	30
Figure 4.8 FC counts at WF1 September 4 th - 7 th	32
Figure 5.1 Rainfall and discharge for study period January 1 st - September 15 th	34
Figure 5.2 FC counts January 1 st - September 15 th	38

Figure 5.3 SpC at WF1 June 21 st - June 23 rd	40
Figure 5.4 Scenarios of epikarst hydrology for south-central Kentucky.....	42

MULTIPLE STORM EVENT IMPACTS ON EPIKARST STORAGE AND TRANSPORT OF ORGANIC SOIL AMENDMENTS IN SOUTH-CENTRAL KENTUCKY

Sean Vanderhoff

December 2011

67 Pages

Directed By: Dr. Jason Polk, Dr. Chris Groves, Dr. Leslie North, and Dr. Carl Bolster

Department of Geography and Geology

Western Kentucky University

The groundwater in agricultural karst areas is susceptible to contamination from organic soil amendments and pesticides. During major storm events during 2011, dye traces were initiated using sulphorhodamine-B, fluorescein and eosine in a groundwater recharge area where manure was applied to the ground. Fecal coliform samples were collected from significant storm events from January-September 2011. Water samples and geochemical data were collected every four hours before, during, and between the storm events from a waterfall in Crumps cave flowing from the known recharge area to track the transport and residence time of the epikarst water and organic soil amendments during variable flow conditions. Two dataloggers at the same waterfall were set up to collect 10-minute data, which included pH, specific conductivity, temperature, and discharge. Total rainfall amount and other surface meteorological data were collected from a rain station located above the cave. Cave water samples were collected for the analysis of anions, cations, bacterial count, and the presence of dye. The dye traces show variability in the characteristics of epikarstic response and flowpaths. The changes in geochemistry indicate simultaneous storage and transport of meteoric water through

epikarst pathways into the cave, with rapid transport of bacteria occurring through the conduits that bypass storage. Fecal coliform counts were elevated all through the study period indicating survivability in soils through the seasons. The results indicate that significant precipitation events affect the storage properties and rapidly impact the various pathways and timing of contaminant transport through the epikarst zone, eventually allowing these contaminants to be transported unfiltered in to the groundwater supply. This study shows that current best management practices in karst lands need to be revisited to incorporate areas that do not have surface runoff but where contaminants are transported by seepage into local aquifer.

CHAPTER ONE: INTRODUCTION

Kentucky's subtropical climate and fertile soil provide extensive agricultural lands for row crops. A common agricultural practice in the area is to apply animal waste as an organic soil amendment for soil nutrient enhancement. If these amendments are not completely exhausted through crop utilization, they can become pollutants and enter the groundwater system. In Kentucky, 55% of the land area is characterized by highly soluble carbonate rocks within which karst landscapes form (Currens 2002). The resulting karst landscape/aquifer systems, typically with high permeability, are characterized by the development of features such as sinkholes, caves, and large springs. Because much of the recharge entering these systems moves rapidly under turbulent flow, and in many cases as sinking streams with little physical filtration, groundwater in these karst aquifers is often highly susceptible to contamination from agricultural practices, among other sources of pollution (White 1988, Pasquarell and Boyer 1995, Drew and Holtzl 1999).

These contaminants can affect not only local drinking water, but in moving through karst aquifers, they can travel long distances and be discharged at springs far from the contamination sources (Quinlan and Ewers 1985). Kentucky's groundwater is an important source of drinking water for many residents of the state. Human health risks and ecological impacts on aquatic ecosystems can be associated with high levels of animal waste-related contaminants such as nitrates, phosphates and pathogenic bacteria.

Best Management Practices (BMPs) are farming methods that aid in maximizing crop yield while minimizing contamination. For water protection, the Kentucky General

Assembly passed the *Kentucky Agriculture Water Quality Act* (AWQA) in 1994 (KRS. 224.71-100 through 224.71-140). The purpose of the act is to protect surface and groundwater resources from pollution as a result of agriculture. BMPs have been initiated for the use of nutrients in row crops. In Kentucky, this policy still is unable to address the complexities and heterogeneous nature of the complex karst hydrology and its influence on the transport of contaminants through the system.

In karst regions, such as Kentucky, the epikarst, or subcutaneous zone, is a major storage component of water entering our aquifers (Williams 1983, 2008; Frederick and Smart 1981; Lee and Krothe 2001; Worthington 2003). Meteoric water from the surface passes through this zone before entering major conduits in the bedrock below on its way through the aquifer. Studying the hydrology and transport of contaminants of the epikarst zone are important to determine the fate of contaminants. Geochemical analysis and tracer tests for storm event monitoring of contaminant transport are important tools to better understand the processes governing contaminant transport under different hydrologic conditions (Göppert and Goldscheider 2007)

This research is designed to better understand the fate and transport of agricultural contaminants in the well-developed karst aquifer/landscape systems of south central Kentucky by conducting field experiments associated with actual field-scale agriculture at the Crumps Cave Educational Preserve, and aims to answer the following research questions: (1) if manure influences aquifer recharge at this representative site, is there significant retardation of flow and storage of water and/or fecal bacteria in the soil/epikarst zone before it enters the main part of the aquifers?; and if so 2) what is the timing of flow through this shallow part of the flow system?; 3) how does that effect the

introduction of fecal bacteria into the main part of the aquifer?; and 4) where is the primary storage for contaminants and bacteria in the soil-epikarst setting? While studies such as Mitchell *et al.* (2005) and Ham *et al.* (2009) have shown major storm and flooding events have a strong effect on transporting nutrients, they do not reveal how individual storm events and seasonal changes effect the residence time of contaminants. The question remains as to the seasonal influences on contaminant transport. This research aims to help understand how storm events of different magnitudes and seasonal changes affect the fate of contaminants as they move through the soil and epikarst zones. Developing a clearer understanding of these processes, in turn, can inform development of BMPs for manure application in row crop farming on karst systems.

Utilizing Crumps Cave in south-central Kentucky, this study helps to identify the transport mechanisms and residence time of bacteria in agricultural settings. The location has morphology typical of the extensive karst “sinkhole plain” landscapes of Kentucky’s Mississippian Plateau that provide some of the state’s most useful agricultural land (Currens 2002; Groves *et al.* 2006). Having access to an integrated, well-characterized study site is the optimum approach to studying karst hydrogeology and is the foundation to developing meaningful models that can be used to test hypotheses of karst flow and transport of contaminants in other, less well-characterized settings (Brahana *et al.* 1999). Performing tests at specific sites, researchers can draw a better picture of contaminant transportation and test to see if current BMP’s are work well in a karst landscape.

1.1 Examination of Epikarst

In most karst regions, carbonate minerals such as calcite (CaCO_3), and less commonly dolomite ($\text{CaMg}(\text{CO}_3)_2$), dominate the geology. In pure waters they dissociate into their constituent ions ($\text{Ca}^{2+} + \text{CO}_3^{2-}$ or $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{CO}_3^{2-}$). When CO_2 gas comes into contact with water, the CO_2 will dissolve until equilibrium is reached. This dissolved carbon dioxide in water is mostly in the form of carbonic acid (H_2CO_3). Rainwater is in equilibrium with CO_2 , but the gases in soils contain typically many more times the amount of CO_2 as a consequence of root respiration and decay of organic matter (Bakalowicz 2003). As rainfall percolates through soil its CO_2 content increases, thus increasing the amount of carbonate rock that can be dissolved by the carbonic acid created in this process (Drever 1988). The epikarst, also known as the subcutaneous zone, is composed of highly weathered carbonate bedrock immediately beneath the surface or beneath the soil where present. It gradually gives way to the unsaturated vadose zone of less weathered bedrock. It can only be seen at the surface where rock outcrops are present (Klimchouk 2000, Jones *et al.* 2004, Groves *et al.* 2006, Williams 2008). The epikarst differs from the rest of the vadose zone by its variable storage capacity, highly variable void distribution, and the dynamic flow of water within it. The high porosity and permeability of the epikarst originates because an increased amount of carbonate rock dissolution occurs close to the soil interface where CO_2 production is greatest (White 1988, Kaufmann & Dreybrodt, 2007, Nguvet *et al.* 2010, Faimon *et al.* 2012). This creates a network fissure system wherein percolating waters widen passages close to the surface but decreases with depth. Porosity in the epikarst can exceed 20% and decrease to <2% in the less weathered vadose zone below (Ford and Williams 2007).

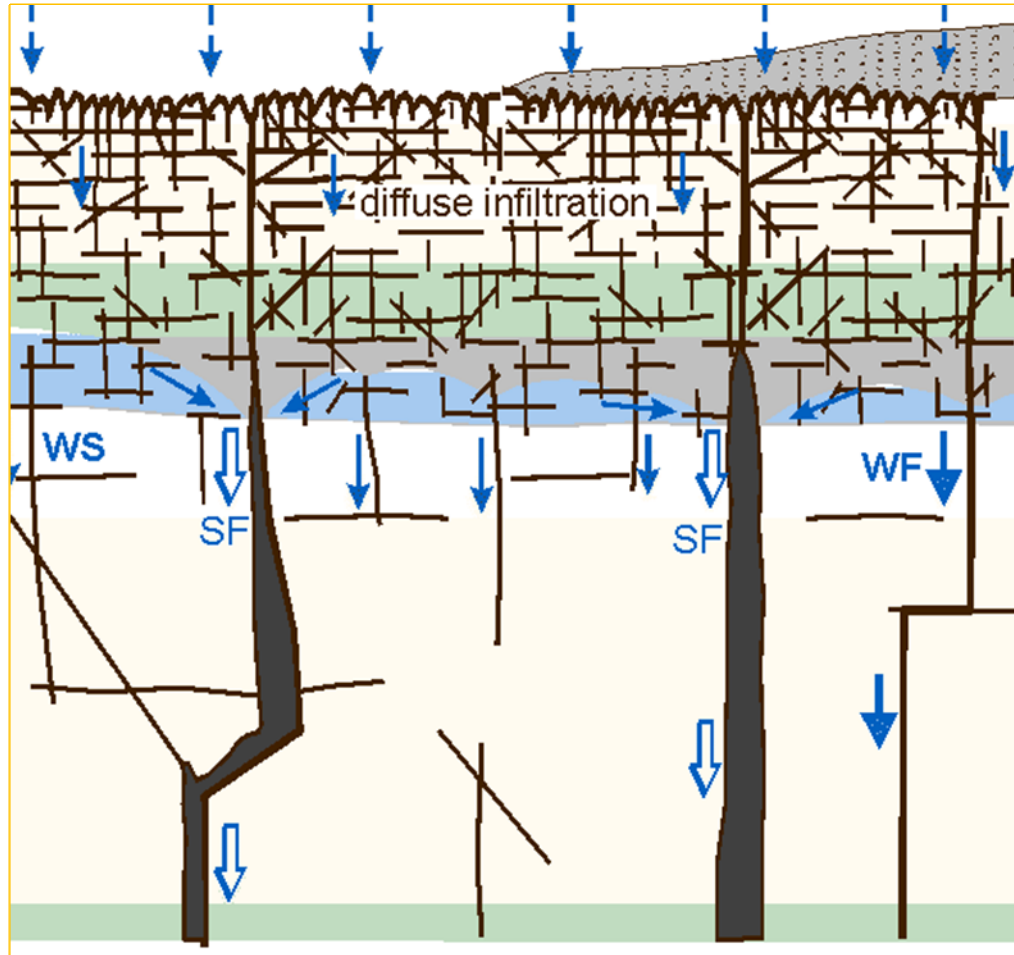


Figure 1.1 Epikarst model by Klimchouk (2004) showing the movement of water in the epikarstic system. Meteoric waters first have diffuse infiltration in the upper portion of the soil - epikarst zone. These waters are either stored or move laterally. Next, waters move rapidly by shaft flow (SF) or are stored and move slowly by vadose flow (WF) or vadose seepage (WS).

Because of this tightening of fissures with depth, water is forced to drain laterally to the few fissures that reach deep in the bedrock (Klimchouk 2004). Water moving downward through these “epikarst drains” often forms waterfalls or may be slow seepages that feed speleothems, depending on the saturation state of the water with

respect to calcite. Klimchouk (2004) noted that the hydraulic conductivity is homogeneous in the top of the epikarst, which allows diffuse infiltration, and becomes increasingly heterogeneous towards the lower portions. If the amount of recharge into the epikarst exceeds the capacity of the epikarst drain to transmit water down into the main part of the aquifer, excess water is stored in the void spaces. This water is referred to as an epikarstic aquifer (Ford & Williams 2007). The epikarst storage component can either distribute water as base flow or a quick flow component as described by Perrin *et al.* (2003) in their conceptual model of a karst aquifer in Switzerland. Their results indicated that the soil and epikarst sub-systems have an important storage capacity, possibly greater than the phreatic zone. Studies by Frederick and Smart (1981) and Lee and Krothe (2001) indicate close to half of all karst water storage may be in the epikarst.

1.2 Fecal Bacteria Survivability in Soil and Water

Since there are a large number of specific pathogenic bacteria in animal waste, the most common way to trace these bacteria in the soil is to measure fecal coliform as an indicator of presents. Generic *Escherichia coli*, (*E. coli*) is the most common indicator of presents and since it is usually not found in natural settings, can be used with fecal coliform to determine the presence of human or animal waste (Crain et al. 1981).

The principal factors in survivability of enteric bacteria are moisture, temperature, nutrients, competition, and soil type. Soil moisture may be the most important factor in determining the survival of enteric bacteria. Research shows higher mortality rates correlate with drier soils and higher survival rates when soils are moist (Crane and Moore

1986; Gagliardi and Karns 2000; Mubiru *et al.* 2000; Nicholson *et al.* 2000; Saini *et al.* 2003; Jamieson *et al.* 2004) Simulating major rain events in soil, Tate (1978) and Saini *et al.* (2003) found *E. coli* survived greatest in flooded conditions, and Hagerdon *et al.* (1978) found *E. coli* populations highest after a rise in the water table following a simulation of major rain events. However, too much moisture in the soil can leave the nutrients unusable (Chandler and Craven 1980).

A majority of the existing research shows an inverse relationship between temperature and survivability (Gerba *et al.* 1975, Jamieson *et al.* 2003, 2004). Van Donsel *et al.* (1967) found a 90% reduction in 3.3 days in summer to 14.3 days in autumn. Reddy *et al.* (1981) noted that die-off rates increases twofold for every 10°C rise in temperature. Nutrients, in the form of organic material found in soils, supported survivability and possible regrowth in some cases (Gerba *et al.* 1975).

Organic matter provides a carbon source and can aid in retention of moisture. Higher mortality rates in subsoil as opposed to topsoil may be due to low availability of nitrogen (Zhai *et al.* 1995). There may also be competition with resident bacteria that can impact the survival of enteric bacteria. Resident bacteria are more resistant to enteric bacteria (Ellis and McCalla 1976, Reddy *et al.* 1981). However, in sterile soils the survival rate increased and sometimes regrowth took place (Tate 1978).

Soil type can influence both transport time and soil retention properties, which are linked to particle size and organic matter distribution. Fecal bacteria moves more rapidly and retains less water in coarse grain sizes, such as sand and larger (Hagerdon *et al.* 1978, Tate 1978, Chandler and Craven 1980, Jamieson *et al.* 2004, Saini *et al.* 2003).

Meteoric waters and runoff, along with the transport of sediment, are the major movers of enteric bacteria in soils. Physical movement through soil is the primary mode of transport of bacteria (Nicholson *et al.* 2000, Tyrrel and Quinton 2003, Jamieson *et al.* 2004). Schwartz *et al.* (2008) showed in an agricultural karst setting that recharge through the epikarst is highly dependent on sufficient precipitation and infiltration over the winter months followed by continued precipitation in the spring.

Additionally, accumulative application of organic soil amendment can add to the survivability of bacteria. Gerba *et al.* (1975) described survival times of enteric bacteria in soil and groundwater that varied from 2 to 4 months. Filip *et al.* (1988) observed *E. coli* to survive for over 100 days at 10°C.

1.3 Best Management Practices

The *Kentucky Agriculture Water Quality Act* crops BMP section 4.5 focuses on nutrient management. Nutrient management is part of the *Agriculture Water Quality Plan* that involves carefully monitoring all aspects of soil fertility and making adjustments so that crop nutrient needs are met while minimizing the loss of nutrients to leaching. This plan includes understanding crop nutrient needs, pH and nutrient testing for soil and water, testing of manure for nutrients, use of cover crops and timing of application

The landowner has prime responsibility for preparing an agriculture water quality plan that best meets the needs of the farming operation. This plan belongs to the landowner, but must be available in the event that water pollution occurs and is identified and traced to the agricultural operation.

Current BMPs due not take into consideration in the processes of the soil - epikarst relationship and the seepage of contaminants contributes more than surface runoff. This research is to inform this relationship to farming operations and lead to the development of BMPs that will help mitigate contamination of this system and assist farmers in meeting its BMPs goals.

CHAPTER TWO: STUDY AREA

Fieldwork for this study was undertaken at the Crumps Cave research site in northern Warren County, Kentucky. Formally known as Cave Springs Caverns, this cave was previously used as a show cave. The site has been owned and operated by Western Kentucky University since 2008. With the area being typical of a karst sinkhole plain located in Kentucky, controlled experiments can be carried out under natural conditions and agricultural practices similar to those found throughout the karst region of south-central Kentucky.

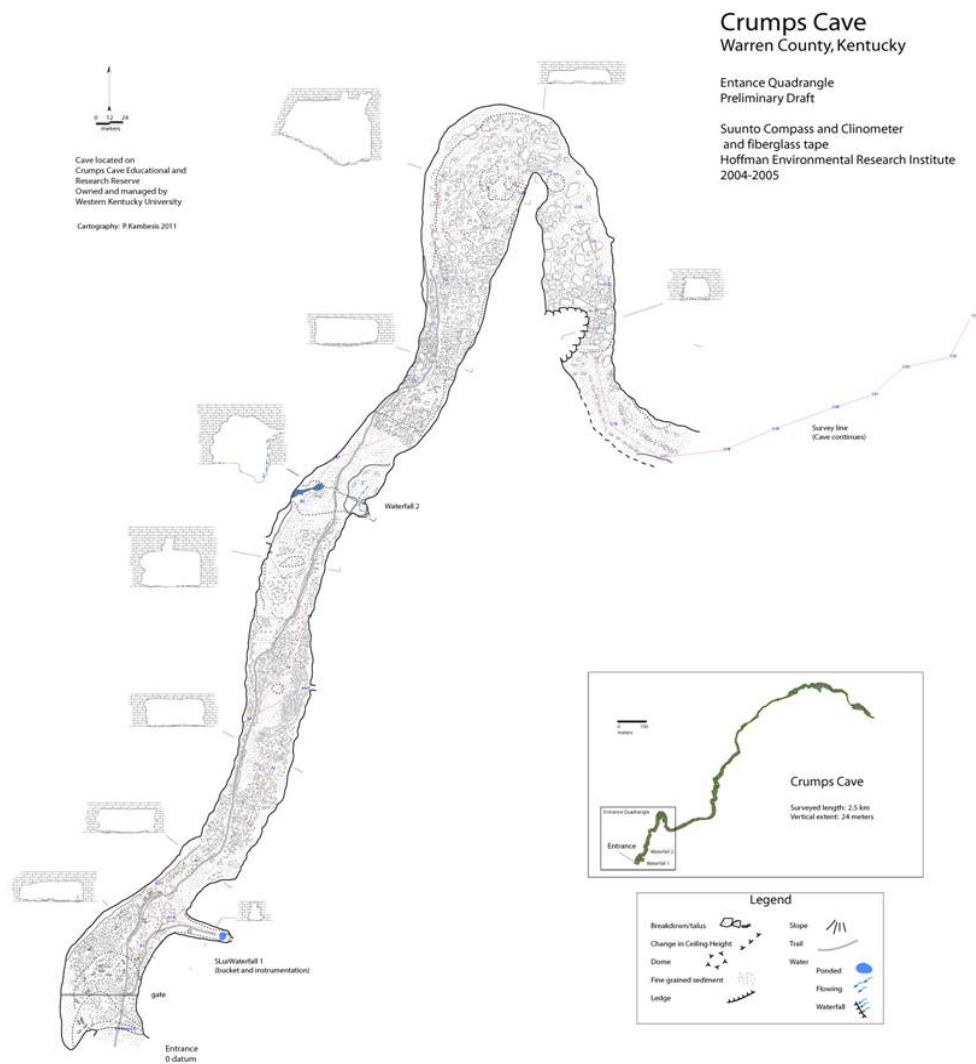


Figure 2.1 Plan view cave map of a section of Crumps Cave. Several epikarstic drains discharge as waterfalls within the cave. Map courtesy of Pat Kambesis and the Hoffman Environmental Research Institute.

Crumps Cave is located beneath a portion of the extensive sinkhole plain of the Pennyroyal Plateau within the Mississippian Plateaus Section of the Interior Low Plateaus Physiographic Province (Groves *et al.* 2005). There is about two km of

horizontal cave passages beneath several agricultural fields, with the cave floor averaging 25 m below the surface. The recharge area lies within the Graham Springs groundwater basin (Ray and Currens 1988, 2000) which discharges at Wilkins Bluehole on the Barren River, 18 km southwest. It is the second largest spring in Kentucky (Ray and Blair 2005). The site is underlain by Crider silt loam, Pembroke silt loam and Baxter gravelly silt loam soils (Soil Survey Staff NRCS 2011). These soils are moderately permeable, well-drained soils, reddish in color with chert fragments in their lower portions. The thickness of the soils varies throughout the study area. Auger hole tests show the thickness before encountering chert fragments ranges from 15 - 72 centimeters.

The entrance to Crumps Cave is a collapse sinkhole that has partially collapsed. The cave passages have formed within the highest part of the Mississippian-aged St. Louis limestone, with a local dip of 1-2° to the west (Richards 1964). The bedded Lost River Chert lies between the ground surface and the cave below, and locally appears to operate as a leaky perching layer. Water tends to reach the cave at distinct locations, mainly as perennial or intermittent waterfalls emerging from the cave ceiling through fractures, draining the epikarstic zone to the east of the cave and flowing westward down the dip of the rock (Bolster *et al.* 2005). Six perennial in-cave waterfalls are located within the entrance area of the cave. These waterfalls are focused on the east side of the cave, but some flow from different parts of the ceiling. Waterfall One (WF1) is approximately 4.5 m tall and is located 40 m from the entrance. It is the closest waterfall to the entrance and has perennial flow (Figure 2.1). It is the focus of the monitoring and research described herein.

The climate of Warren County is classified as a humid subtropical climate on the Köppen climate classification scale (*Cfa*). Its humid summers reach an average high temperature of 31°C and its mild to cool winters average a high of 7°C (NOAA 2011). The average annual total precipitation is around 1294 millimeters. Of this, about 721 millimeters, or 56 percent, usually falls in April through October. May has the highest average rainfall with 136 millimeters (NOAA 2011). The growing season for most crops falls in the April through October range. Hess (1974) estimated that mean-annual potential evaporation is 800 mm, varying from near zero to over 100 mm/mo.

Land use above and surrounding Crumps Cave is dominated by agriculture (Figure 2.2). Row cropping, which usually rotates between corn, soy, and wheat, surrounds the Crumps Cave property to the east and north. West of the property is a residential property at which a bed and breakfast operation is run. Northeast of the property land is currently being used for cattle grazing.

Crumps Cave was used as a local water source for generations. Pipes at certain perennial waterfalls inside the cave would carry pumped water up to the surface for domestic use. These pipes are not currently in use, but still remain at some of the waterfalls.



Figure 2.2 Crumps Cave overlay and surrounding agricultural fields. Map created from data provided by the Kentucky Division of Geographic Information and Hoffman Environmental Research Institute.

CHAPTER THREE: METHODOLOGY

On the surface at Crumps Cave, 110 meters from the sink entrance, a HOBO U-30th weather station was used to collect weather data. A rain gauge tipping bucket collected rainfall amounts every ten minutes. The weather station also collected temperature, dew point, solar radiation, relative humidity, wind speed and direction, and soil moisture content with ten-minute resolution.

Inside the cave, a 208 liter barrel with circular holes drilled into its side to measure discharge was placed under WF1 and a conical tarp directs virtually all flow of the epikarst drain into the barrel. A procedure based on Bernoulli's law relates the WF1 discharge rate (L/s) to the water level (stage height) in the barrel. The water level is measured by a pressure transducer inside a stilling well at ten-minute resolution. Four different sized holes were drilled in the barrel to allow for discharge measurements over three orders of magnitude. In ascending order, these holes represent 0.05 L/s, 0.56 L/s, 5.66 L/s and 8.49 L/s (Figure 3.1). Units are converted to L/s for final discharge measurements. To determine the strength of the correlation between stage height and discharge during low flow periods a manual calibration of discharge was performed for the bottom hole in the barrel (0.05 L/s) by timing how long it took to fill a 4.0 L bucket from the hole. Discharge measurements from the manual measurement were compared to the discharge measurements calculated from the barrel equation during baseflow using linear regression. This allowed for calibration of the equation used to calculate the discharge measurements from stage height. Another linear regression was plotted

between stage height and the calculated barrel discharge measurements to calibrate the equation with the stage height readings as well.

Tracking soil and epikarstic water movement through a karst system can be difficult and expensive through seasonal changes over long periods. Because of this, many studies have used specific conductivity, pH, and temperature to develop chemographs for following waters through soil and karst pathways (Bakalowicz 1979, Hess and White 1988, Ryan and Meiman 1996, Grasso *et al.* 2003, Birk *et al.* 2005, Groves and Meiman 2005; Toran *et al.* 2006, Raeisi *et al.* 2007). Temperature, pH and specific conductance were measured *in situ*. Interpretation of these parameters can provide an idea of the residence time of epikarstic waters.

At WF1, two Campbell Scientific CR10x data loggers were used to collect geochemical and discharge data for the waterfall. Data Logger One (DL1) recorded data from one pH probe, one dual specific conductance and temperature probe, and a pressure transducer probe placed in the discharge barrel at WF1. Data Logger Two (DL2) recorded data from two pH probes and a dual specific conductivity probe. Both data loggers collected data every two minutes and recorded the average every ten minutes for temperature, SpC, and pH. Stage height from the pressure transducer was also recorded every ten minutes.



Figure 3.1 Discharge barrel and data logger set up at WF1 during spring storm event.

Continuous ten minute resolution data were collected from January 1st, 2011 (Julian Date (JD) 001) to the end of the study period September 17th (JD 260). The study period was chosen by the first storm event after observing area farmland had application of organic soil amendments on the ground (applied the last week of December). This time period also covers the pre- and post-growing seasons for the area. Weekly calibration of the three pH probes was conducted in pH buffer four, seven, and ten. This calibration was

performed to find a standard deviation of the three pH data probes. Mean temperature and SpC was found by averaging the two probes. The data from the CR10x dataloggers were transferred by a Campbell Scientific CR10KD keyboard display. The data from the CR10KD dataloggers were transferred to spreadsheets and analyzed using Sigmaplot 11.0. Discharge data and weather data were added to the spreadsheet along with *E. coli* and FC counts.

During the farming season of late winter through spring, three fluorescent dye traces took place to track transport and residence time of water from storm events and epikarstic waters. The dyes were chosen for their spectrum wavelength so as to be able to recognize each individual dye as it came through WF1 from the surface. The traces were performed in a location on the edge of the property in an area that has previously been established as having a hydrological surface connection to WF1.

ISCO 3700 portable water samplers were placed in the cave at WF1 to collect water samples to analyze for dye, bacteria, cations and anions. Samples were collected in 1000 mL polypropylene bottles every four hours during storm events occurring within the study period. During a portion of the winter and spring sampling period weekly samples of FC were taken. Samples were also collected weekly for the analysis of dye and collected within 24 hours of analysis time for total coliform, *E. coli*, cations and anions. Cation and anion samples were then pipetted into 25 mL polypropylene centrifuge bottles and sent to the WATERS Laboratory at Western Kentucky University for analysis. FC samples were sampled from the ISCO's or directly from the waterfall into sterilized 50 mL polypropylene containers and sent to the WATERS Laboratory within 24 hours of collection. Total Coliform and *E. coli* samples were analyzed using the Colilert MPN

method. A Colilert-18 and Quanti-tray 2000 were used to enumerate *E. coli* in source water pursuant to the Long Term 2 Enhanced Surface Water Treatment Rule (WATERS Laboratory 2011). After the appropriate sample dilutions/volumes were added, the trays were incubated for 18 hours at ~44.5° C. Each well was then compared to the reference color comparator available from the manufacturer. A yellow color greater or equal to the comparator indicates the presence of total coliforms in the sample. The total coliform wells were then checked for fluorescence under long-wavelength UV light (365-366nm). A yellow well with fluorescence greater than or equal to the comparator is positive for *E. coli*. The most probable number (MPN) value was determined by the number of positive wells using MPN tables provided by the manufacturer. *E. coli* densities are then calculated and reported as MPN/100mL. If there were any uncertainties associated with a well as to whether or not the well is positive, the tray was placed back into the incubator for four hours to see if the result becomes more pronounced.

3.1 Fluorescent Dye Tracing

Dye for the first trace was injected on February 1st (JD 032) and used 0.68 kg of dry powder Sulphorhodamine-B was mixed with 9.46 liters of water and applied to 5 auger holes drilled into the top 16 centimeters of the soil. An additional 9.46 liters of water were used to wash out the dye container and applied to the 5 auger holes dug in a location previously established as hydrologically connected to WF1.

The second dye trace was initiated on February 23rd (JD 054) using 0.49 kg of dry powered fluorescein mixed with 9.46 liters of water and injected in the same 5 auger holes as the SRB trace. An additional 9.46 liters of water was added to the holes during

the trace. The third dye trace used 1.81 kg of dry powdered eosine mixed with two 9.46 liter containers of water. The third dye trace occurred on April 25th (JD 115) using 1.81 kg of dry powdered eosine mixed in two containers of 9.46 liters of water and applied to the 5 auger holes.

Prior to the dye injections activated charcoal packets were placed at the six perennial waterfalls, one intermittent waterfall, and the dripline for detection of dye. A set of the packets was left in the cave for one week, replaced and analyzed for dye prior to the first injections to ensure that no high background levels of fluorescence were present that could interfere with interpretation of tracing results. The charcoal packets were collected weekly for the entire study period and refrigerated at 3.3°C until analysis.



Figure 3.2 Injection of eosine on April 25th, 2011.

3.2 Analytical methodology

During the dye traced storm events, the automatic water samplers collected samples from WF1 every four hours. Samples of 10 mL, from the automated samplers at WF1, were collected in glass vials and kept cooled at 3.3°C until analysis for dye. The water samples were analyzed using a Shimadzu RF 5301-PC spectrofluorometer (Shimadzu Scientific Instruments Inc., Columbia, MD) at the Crawford Hydrology

Laboratory at Western Kentucky University and results reported in parts per billion (ppb) according to standard lab protocol. The charcoal packets were washed, sample size weighed out and eluted with a solution of 50% N-Propyl Alcohol, 30% de-ionized water, 20% NH_4OH and then analyzed using the Shimadzu RF 5301-PC spectrofluorometer (Shimadzu Scientific Instruments Inc., Columbia, MD). Emission and excitation wavelengths specific to the dyes that were analyzed were tested by the machine as a light passed through the sample. A curve produced by the program could be ruled a positive or negative result for a certain dye based on where the peak was in the emission spectrum. The shape and magnitude of the peak were used to determine the concentration of a given dye within the sample. These concentrations were then recorded in a spreadsheet in parts per million or parts per billion based on sample concentration.

CHAPTER FOUR: RESULTS

4.1 Dye Trace Storm Events

The dye sulphorhodamine B for the first trace was injected on February 1st (JD 32). The storm associated with the dye trace totaled 23 mm of rain over 48 hours (Figure 4.1). The base flow prior to the storm at WF1 averaged 0.11 L/s with a minimum discharge of 0.10 L/s. The peak flow of discharge during this storm event was 1.90 L/s on February 1st. Discharge returned to previous base flow levels on February 8th (JD 39).

The SpC levels prior to the storm event at WF1 measured 217 $\mu\text{S}/\text{cm}$. The SpC dropped on February 1st to 157 $\mu\text{S}/\text{cm}$ indicating infiltration of meteoric water at WF1 within one hour. SpC slowly recovered to pre-storm levels by February 10th (JD 41) indicating a relaxation time of about ten days under these conditions.

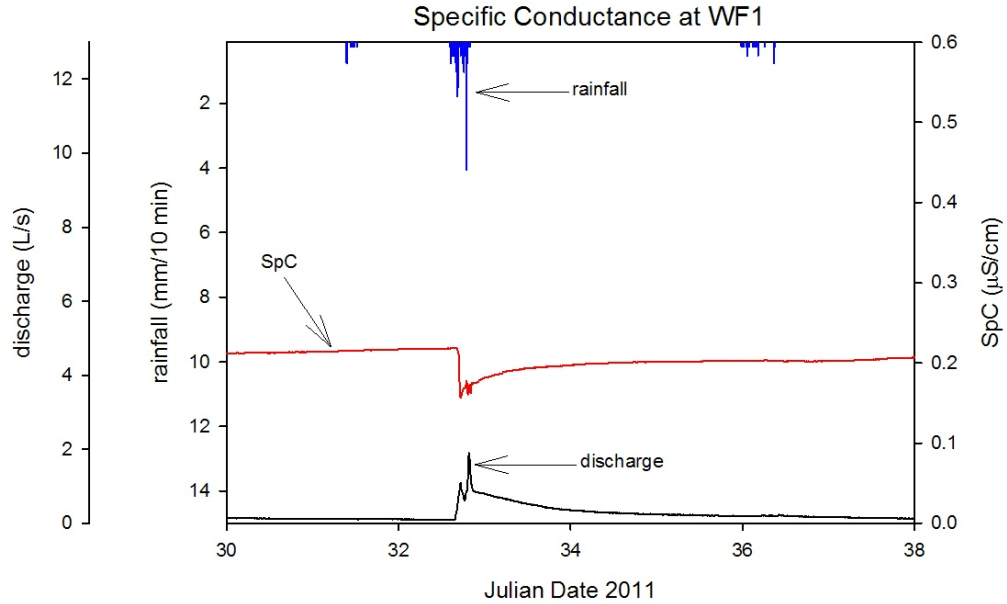


Figure 4.1 SpC data at WF1 January 30th - February 7th

Fecal coliform (FC) levels prior to the injection storm were relatively low, typically with counts of 1-2 colonies/100mL. FC increased sharply with the rise correlating with the increase in discharge. FC counts rose from 10 to 8.1×10^2 colonies/100mL on February 1st (Figure 4.2). Samples eight hours later after discharged dropped measured 1.1×10^2 colonies/100mL. *E. coli* samples were also collected along side FC. These counts mirror those of FC, including the patters of concentration fluctuation, during this and most other storm events sampled during the study period. Because FC is an indicator of *E. coli*, FC counts are used in the remaining results.

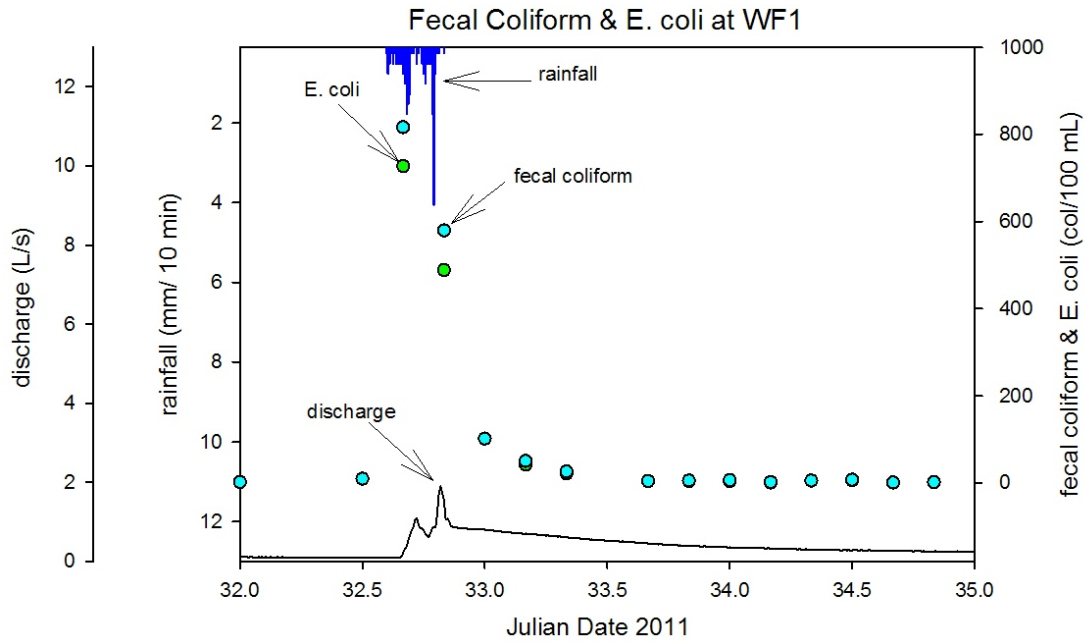


Figure 4.2 FC and E. coli counts a WF1 January 30th - February 7th

Sulphorhodamine B was not detected during the storm or directly after the storm of February 1st - 3rd. Two samples positive for Sulphorhodamine B were detected with storm events on February 25th and February 28th (Figure 4.3).

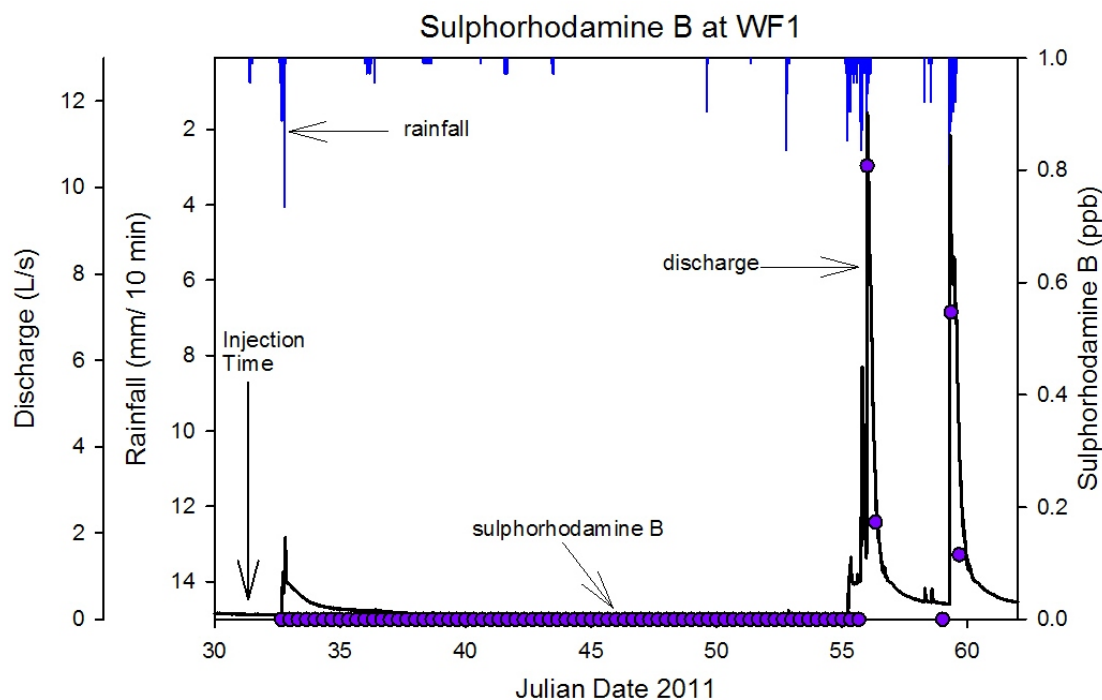


Figure 4.3 Sulphorhodamine B at WF1 February 1st - March 1st

The second dye trace was initiated on February 23rd (JD 54). This injection storm totaled 79 millimeters of rainfall over 48 hours. Base flow for WF1 averaged 0.06 L/s with a low discharge of 0.05 L/s on February 23rd. The rain event started on February 24th (JD 55). Fluorescein passed through WF1 beginning less than 12 hours after the storm event started and 30 hours after the initial injection (Figure 4.4). The first peak discharge for this event reached 5.85 L/s on February 24th (JD 55). A second and higher peak for discharge reached 11.72 L/s on February 25th (JD 56). This peak in discharge also correlate with a high peak of fluorescein measured at 8.5 ppb. Two other significant rain events occurred in this period. The second rain event started on February 28th (JD 59) with a discharge of 0.35 L/s and quickly climbed to 11.20 L/s and a peak of fluorescein

for this storm event measured 5.6 ppb. The second rain event totaled 46 millimeters of rainfall. The third rain event occurred on March 5th - 6th (JD 64-65) with a total of 44 millimeters over 48 hours. Base flow discharge before this event was 0.32 L/s and reached a peak of 3.00 L/s on March 5th (JD 64). Lower concentrations of fluorescein were detected until March 8th (JD 67). Base level flow returned to pre-injection storm levels on March 26th (JD 85). Due to the magnitude of the storm response at WF1, disruption of the data logging caused loss of WF1 pH, temperature and SpC data from this storm.

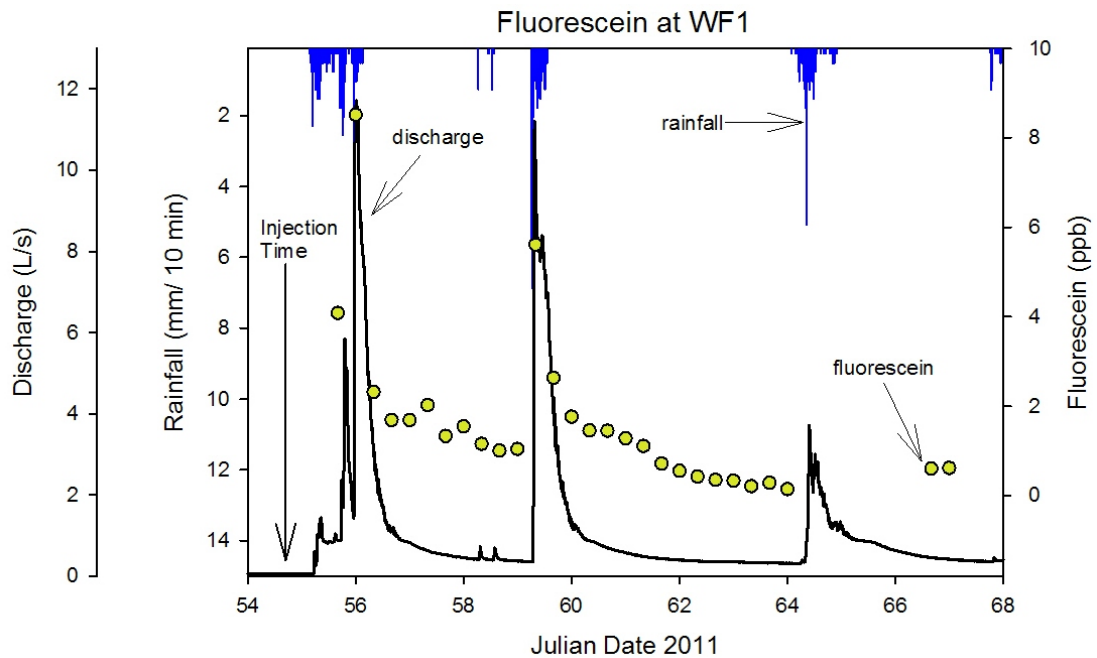


Figure 4.4 Fluorescein at WF1 February 23rd - March 9th

The third dye trace, using eosine dye, was injected on April 25th (JD 115). The injection storm totaled 61 mm, with 33 mm of rainfall occurring in the 24 hours before the injection (Figure 4.5). Discharge for WF1 at time of injection was 0.84 L/s coming down from a peak of 1.45 L/s from the previous storm. The peak discharge for the injection storm reached 9.14 L/s on April 27th (JD 117). A second small discharge peak occurred at May 1st (JD 121) with a discharge of 0.96 L/s. The third peak within the trace period reached at 10.70 L/s on May 3rd (JD 123).

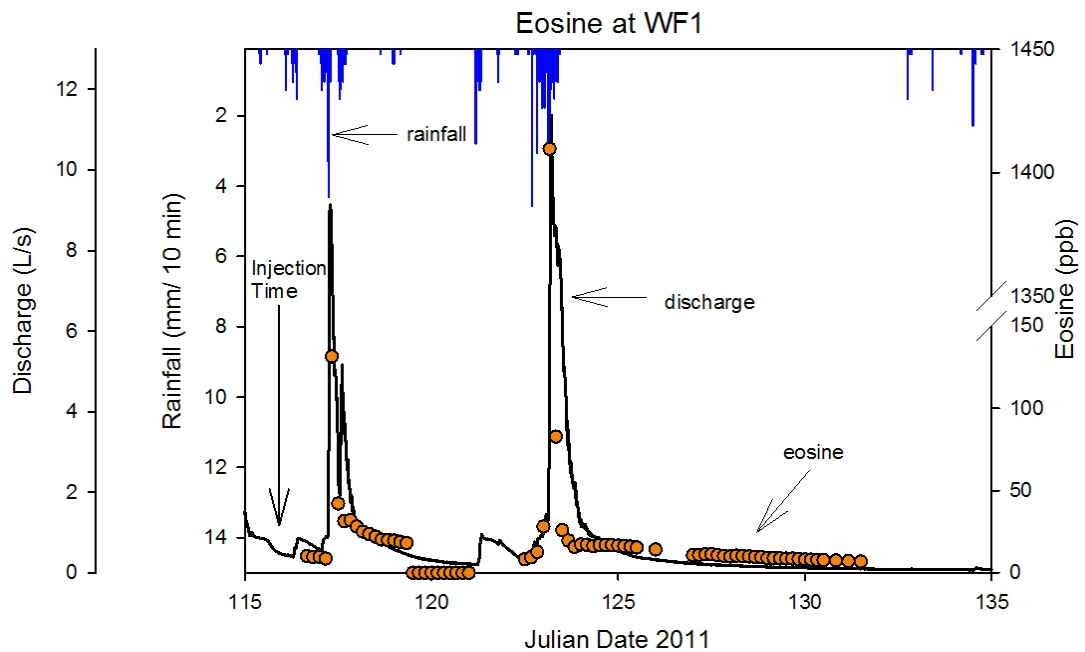


Figure 4.5 Eosine at WF1 April 25th - May 10th

The average SpC prior to the injection was 203 $\mu\text{S}/\text{cm}$ (Figure 4.6). During the injection storm, the SpC dropped to 170 $\mu\text{S}/\text{cm}$ on April 27th and returned to 203 $\mu\text{S}/\text{cm}$

on April 30th (JD 120). This SpC drop is associated with an increase in discharge. SpC at WF1 rose back to 214 $\mu\text{S}/\text{cm}$ on May 2nd (JD 122). A second significant rain event occurred on May 2nd and SpC dropped to 165 $\mu\text{S}/\text{cm}$. SpC again reached 214 $\mu\text{S}/\text{cm}$ on May 10th (JD 130).

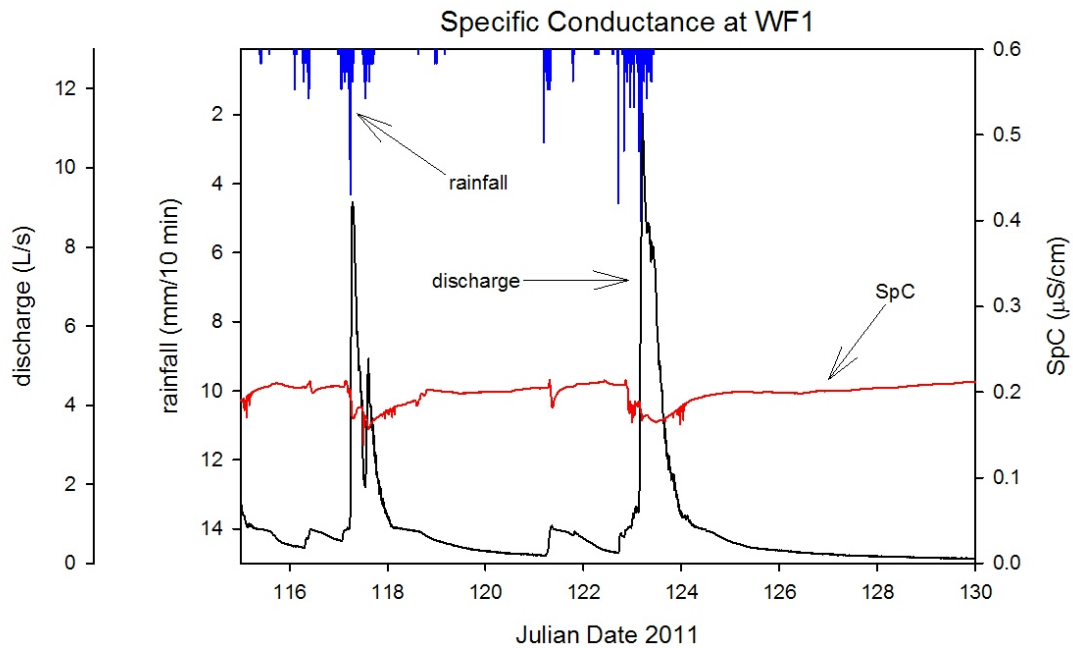


Figure 4.6 SpC at WF1 April 25th - May 10th

FC samples for the injection storm on April 25th (JD 115) contained 1.7×10^2 colonies/100mL and reached 1.1×10^3 colonies/100mL on April 26th (Figure 4.7). Fecal coliform samples reached a peak of 9.6×10^3 colonies/100mL on April 27th (JD 117). Elevated levels of FC were still measured on April 29th (JD 119), with 2.4×10^2 colonies/100mL. Before the May 3rd (JD 123) storm event, FC levels were 1.6×10^2 colonies/100mL. During this storm a peak of 7.9×10^3 colonies/100mL was measured before FC levels dropped to 2.1×10^2 colonies/100mL on May 4th (JD 124).

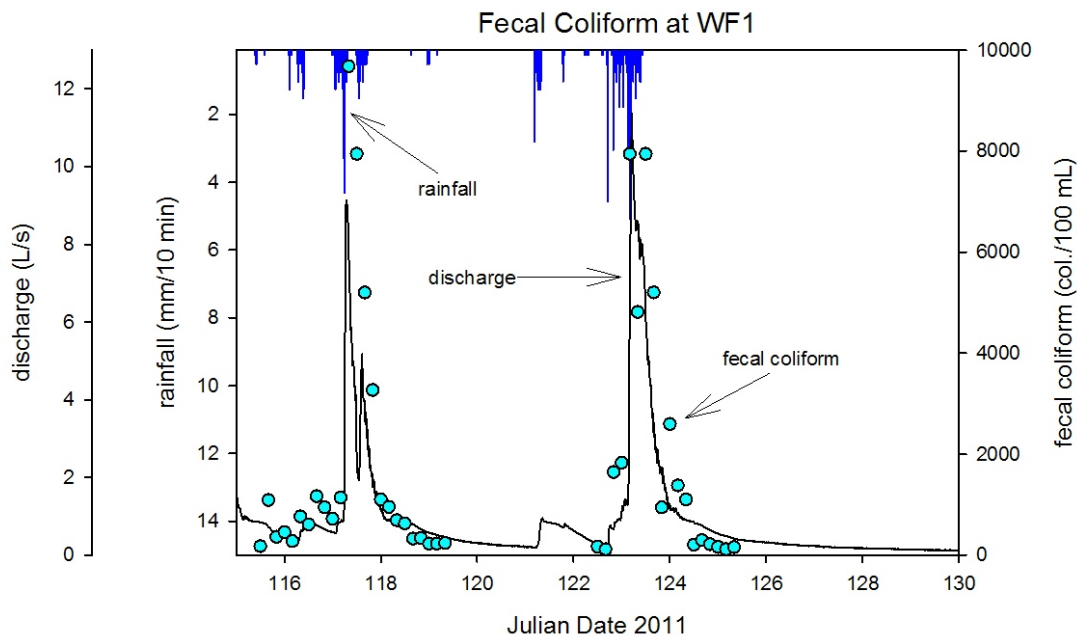


Figure 4.7 FC counts at WF1 April 25th - May 10th

4.2 Seasonal Results

Storm events during the study period all show a pattern related to the parameters measured in this study. The winter months showed fewer large storm events, but the results indicate meteoric waters discharging from WF1 during these events. During the study period, the month of April had record high precipitation both for the state of Kentucky and Warren County. The state average rainfall for Kentucky totaled 302 mm and Warren County totaled 263 mm of rainfall recorded at the Bowling Green-Warren County regional airport weather station. Average rainfall for this area is 107 millimeters for the month of April. The weather station in the study area recorded a total rainfall amount of 264 mm. Rainfall events occurring in this time period had an average discharge of 0.57 L/s.

The April 12th sample was collected during a storm event that measured 92 mm. This event recorded the most rainfall for a single event during the study period. FC and levels for the weekly sample were greater than 2.4×10^5 colonies/100mL. Base flow discharge prior to this storm event averaged 0.09 L/s and quickly climbed to a peak of 9.36 L/s.

The summer months were unusually dry as compared to most years. During the period of July 1st - August 1st (JD 182-243) 58 mm of rainfall was reported. Base flow during the summer months at WF1 reached less than 0.006 L/s, but never went completely dry. The few storm events that did occur observed a drop in SpC and an increase in discharge, likely indicating the input of meteoric water to WF1.

The last FC samples collected during the study period were from a storm event on September 5th (JD 248) (Figure 4.8). Peak FC levels reached at 9.2×10^3 colonies/100mL and quickly dropped to less than 1.5×10^2 colonies/100mL. Base flow prior this storm event reached the lowest levels of the study period. Average discharge was recorded at 0.98×10^{-6} L/s. The peak discharge for this storm reached 0.86 L/s.

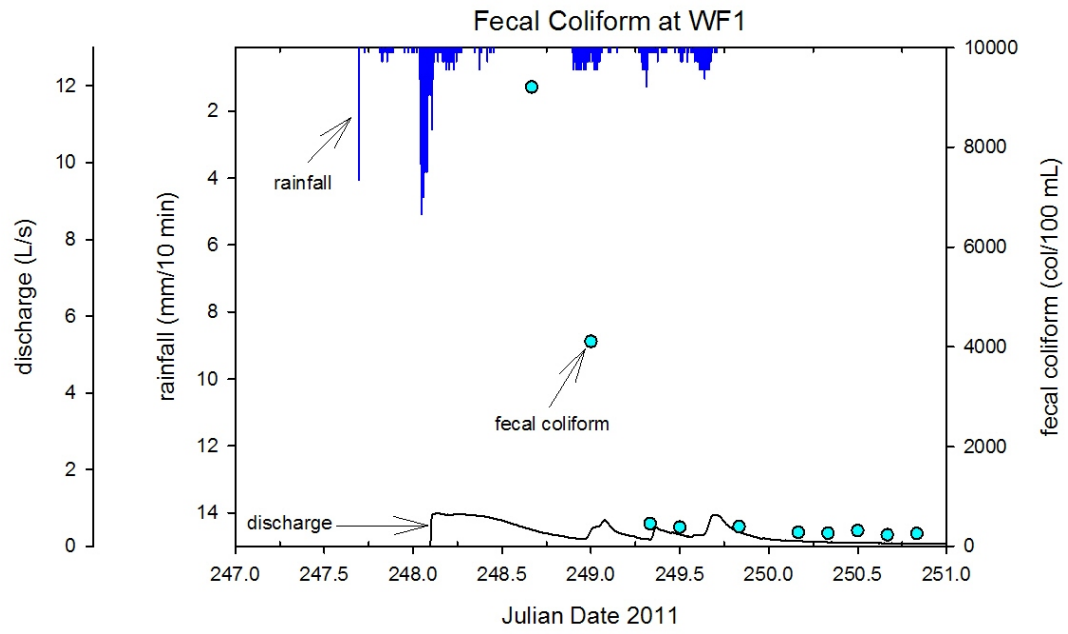


Figure 4.8 FC counts at WF1 September 4th - 7th

CHAPTER FIVE: DISCUSSION

Identifying the hydrological characteristics of the soil-epikarst zone in response to storm events is an important task for understanding the fate of agricultural contaminants. Recognizing the mechanisms of movement and storage is central for determining the fate of these pollutants. The movement of FC through the soil-epikarst is dictated by the amount and intensity of storm events. During the study period, the majority of storm events provoked a response at WF1 indicated by the increased discharge. The data show contaminants that move through the epikarstic system correlate with significant rainfall events and rainfall amount. The dye traces and SpC data add to this statement and further the understanding of soil-epikarst hydrology and contaminant transport.

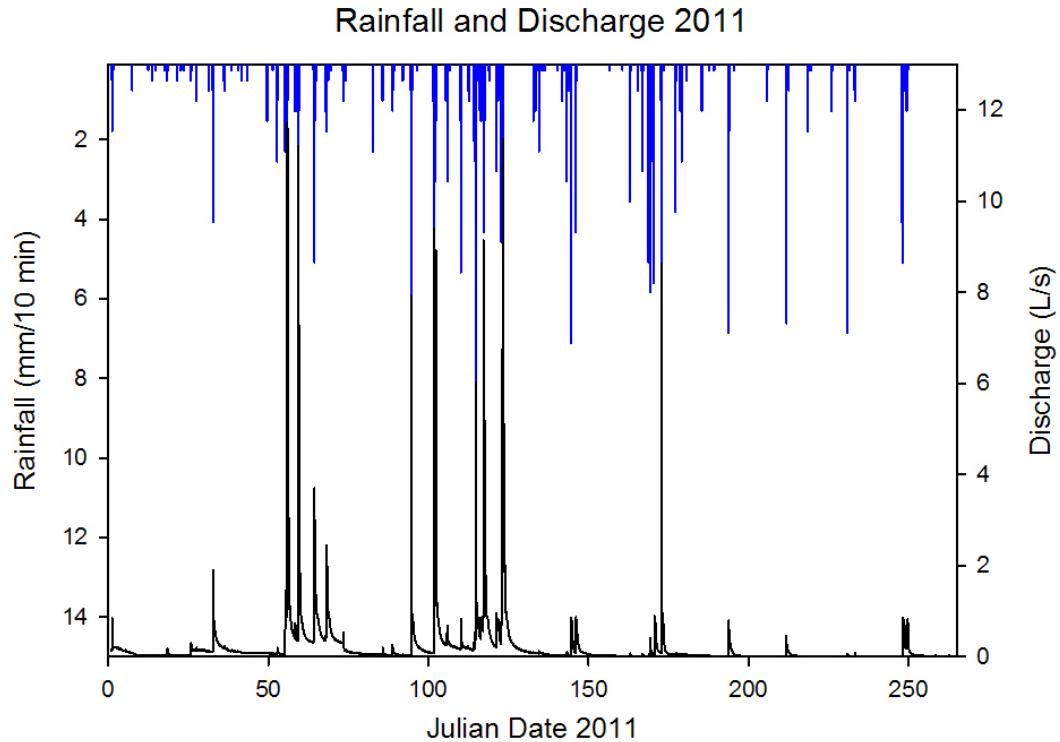


Figure 5.1 Rainfall and discharge for study period January 1st - September 15th

5.1 Dye Traces

The dye traces performed during several storm events of late winter (February 1st and February 23rd) and spring (April 25th) reveal how storm intensity and magnitude and possibly season indicate the hydrological characteristics and how they affect storage and transport of contaminants.

With the first dye trace of sulphorhodamine B, the 23 mm of rainfall may not have met the threshold needed for rainfall intensity and amount to push the dye through the soil-epikarst. With low base flow at the time of the storm event during the SRB trace, some meteoric water probably entered storage and may have pushed existing storage water out. However, the drop in SpC (Figure 4.1) and elevated levels in FC (Figure 4.2)

that occurred with the discharge peak in WF1 indicate that meteoric water did move through the epikarstic system. This meteoric water may have come from movement within the soil-epikarst that allowed for meteoric waters to be discharged at WF1 while the dye was still working its way through the system. The next major storm event did not occur for several weeks and only a total of 55 mm were measured before and after the first trace. This low rain amount shows that the dye may have been added to the depleted epikarstic storage and allowed for lateral movement of the dye within the mature epikarst of the area. This likely explains why only four detections of sulphorhodamine B from this trace and were found during the next major storm event. These data show strong correspondence to the results by Groves *et al.* (2006) and how the epikarst can influence flow and transport of waters in this region. Additionally, it is also possible that the dye may have degraded within the soil and dropped to non-detectable levels.

The second trace of fluorescein totaled 79 mm of precipitation that pushed the dye through rapidly. Fluorescein entered WF1 less than 12 hours after the storm event started. Peak detection of dye correlated with peak levels of discharge and drops in SpC. Detection of the dye was recorded constantly throughout different storm events. Due to the nature of the mature epikarst in the area, the large amount and intensity of rainfall may have both bypassed epikarstic storage completely by a large conduit directly connected to WF1 or pushed storage waters out and flushed part of it through (Klimchouk 2004). SpC data and FC counts not being available for this event, it is important to look at the discharge data closely. Figure 4.4 shows a slight increase in discharge during the beginning of the storm event. The dye at WF1 is not seen until discharge significantly increases with storm intensity and amount. The first increase in

discharge may indicate storage waters being pushed through by meteoric water. The significant increases in discharge may indicate meteoric waters move through the soil-epikarst and gives a plausible explanation for the first dye detection.

The third dye trace of eosine in late April, as with the fluorescein trace, moved through the system rapidly. More precipitation was recorded throughout this trace than the previous trace but the dye took longer to discharge from WF1. This may be caused by the larger amount of dye injected (1.81 kg compared to 0.68 kg and 0.49 kg) during this storm. The peaks of dye correlated with the peak discharges and peak FC counts (Figures 4.5 and 4.7). The first spike in dye detection rose by order of a magnitude from 8.58 ppb to 131.40 ppb between two four-hour samples. This shows the rapid infiltration of dye associated with discharge. Dye levels dropped drastically after the first storm event and again increased with a rise in discharge correlated to storm event. This second storm event, concentration rose from 28.08 ppb to 1409.60 ppb between two four-hour samples. FC counts also rose greatly during this storm event (1.8×10^3 to 7.9×10^3 colonies/100 mL). These large spikes in concentration show movement of dye is dictated by significant storm events. The dye being held in the soil slowly percolating through the system until being pushed through by the rain event. Similar response is seen in the fluorescein trace. SpC measurements also dropped at the times associated with the peaks adding evidence to the movement of meteoric water through the system and soil storage.

5.2 Fecal Coliform Indication

During every major storm event when FC samples were collected either hourly or weekly, the counts were all above the 0 colonies/ mL standard for drinking water and during high precipitation and high discharge events the counts were well above 200 colonies/100mL for contact standard set by the USEPA. Levels of FC detected during the February 1st storm event shared similar counts in FC compared to the September 4th storm, but with less rain (Figure 5.2). The highest counts of FC for the year were associated with the April 11th-12th storm event and are three orders of magnitude higher than the USEPA standards. The majority of samples during the wetter than average April-mid May show peaks an order of magnitude higher than USEPA levels allow. The storm events on April 25th and May 3rd (Figure 4.7) show a good representation of the hydrological characteristics and its affect on transport of FC. Each storm had a peak in FC counts associated with peak discharge and then dropped until the next peak in discharge, which then increased again.

The FC counts from the September 4th-7th (Figure 4.8) samples at WF1 show elevated levels of FC more than 9 months after observed application peak levels associated with the peak discharge. Samples prior to the storm event were not analyzed. There was a peak in FC counts of 9.2×10^3 col/100 mL sampled this storm event. This count appears to be coming off a peak during the falling limb of the storm pulse. These results provide evidence that FC survived the dry summer months and thrived in the subsoil and soil water. These findings are generally consistent with those of Pasquarell and Boyer (1995) regarding the influence of soil moisture on survivability of FC bacteria. The wetter than normal month of April and the fine grain clay loamy soils of the area

both support the claim that the rains helped to replenish soil water and helped to retain nutrients for greater survivability of FC. There is a possibility, though more study is needed, that the soils in the area may also act as an aquatard and prevent the rapid movement of water through them, except for few conduits formed by past storm events.

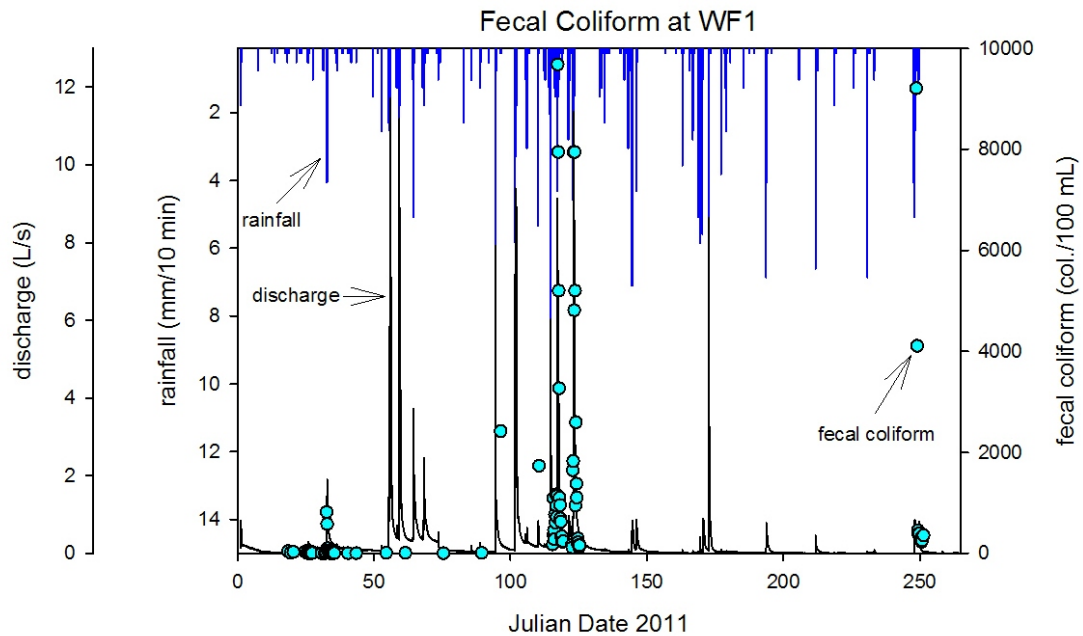


Figure 5.2 FC counts January 1st- September 15th

5.3 Seasonal Storm Events

Storm events and the changes in seasonality during the study period mostly exhibit the same response of increased discharge associated with major rain events (Figure 5.1). The cooler months (January, February) experience similar responses to the warmer months (June, July, August) due to small amount of significant storm events. During the cooler season, some of these storms were very low in amount of rain and did not have a response at WF1. More research is needed to see if winter soil temperatures are playing a role in this or if a threshold for storm amount had not been met. The winter months on average have a lower base level discharge but during significant storm events the SpC drops with responses to rainfall and increased discharge. The summer months were unusually dry for the area, experiencing 6 weeks of almost no precipitation (Figure 5.1). However, the storm events that did take place showed the characteristics similar to those of other storms in different seasons (Figure 5.3)

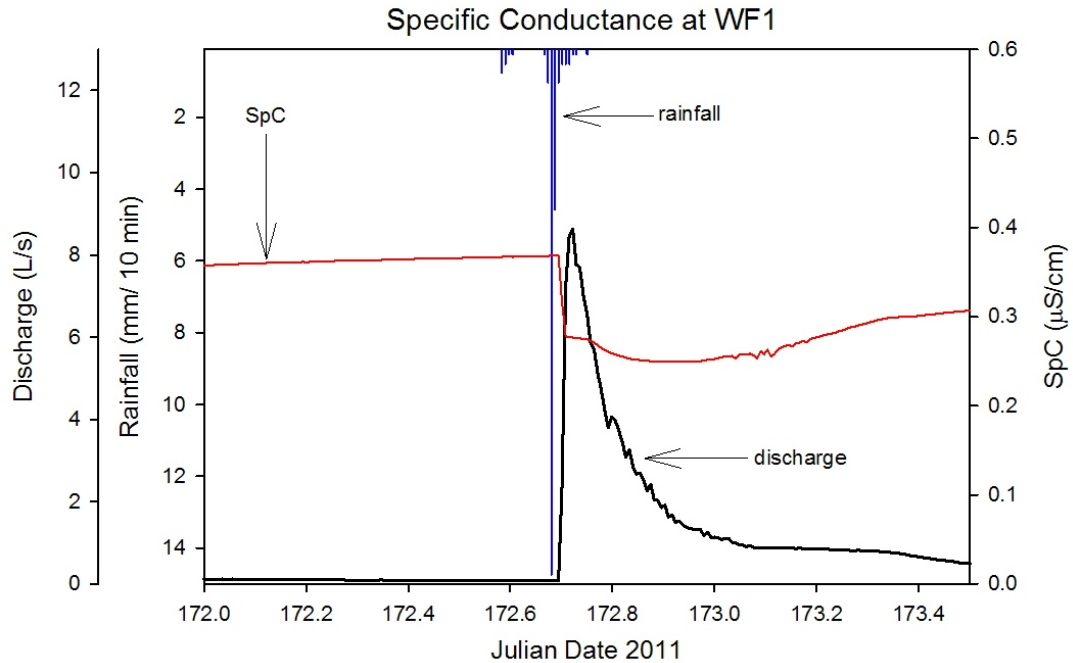


Figure 5.3 SpC at WF1 June 21st - June 23rd

The example of the June 21st storm (Julian Date 172) shows the typical summer response to storm events. The soil may have still been saturated enough to allow meteoric water to infiltrate the epikarst and discharge at WF1. Similar responses of a drop in SpC and increased discharge were seen at WF1 during July- September. The September storm precipitated more than four times the amount of rain as any storm during the warmer summer months (Figure 5.1).

5.4 Possible Scenarios of Hydrological Characteristics of Soil- Epikarst Movement

From this study, four scenarios are possible pertaining to the hydrologic characteristics that dictate the storage and transport of water in the soil-epikarst in south-central Kentucky (Figure 5.4). In the first, water and contaminants are likely stored in the soil for long periods, providing suitable conditions for continued proliferation of the bacteria. The clay soil found in this area does not allow the rapid movement of water and may act as an aquatard that allows for slow percolation of meteoric waters. Second is the classical epikarst storage model (Klimchouk 2004), where the amount of recharge exceeds the vertical flow through and is stored in void space and diffuse conduits. Third, the Lost River chert found in the area could act as a leaky perching layer that deters rapid vertical flow through. In the final scenario, water bypasses storage by way of direct conduits, and discharges out of the epikarst rapidly in WF1.

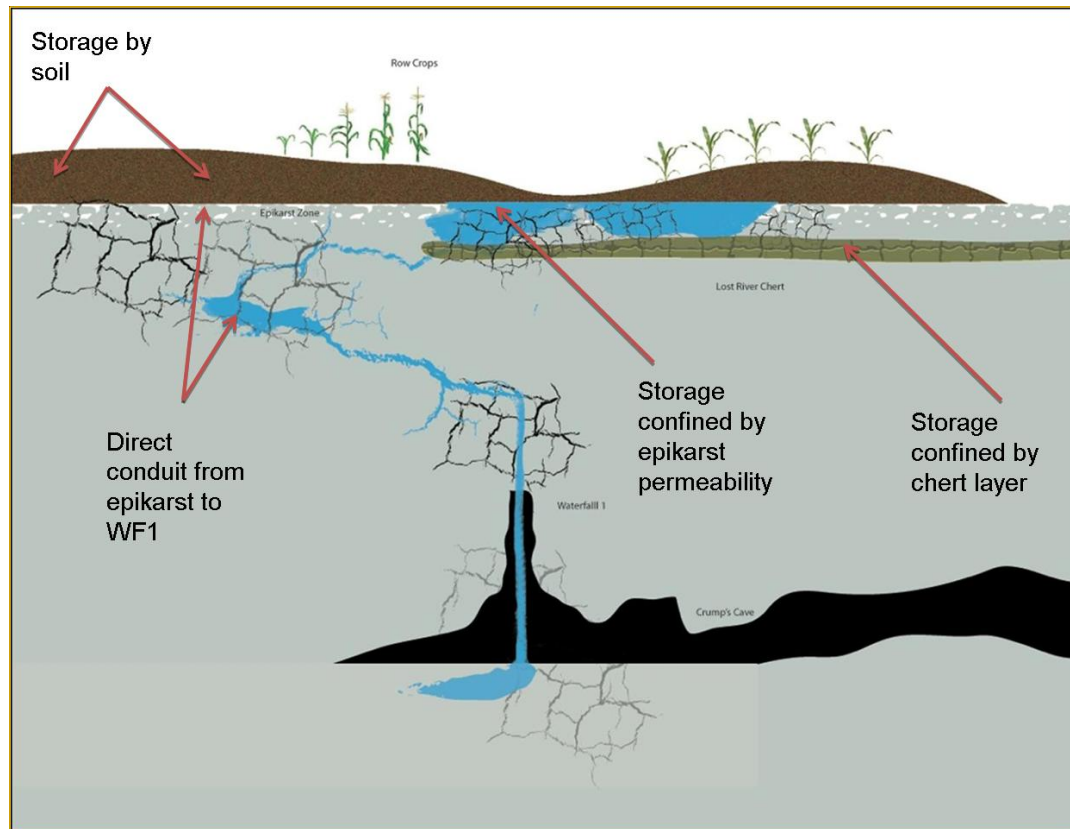


Figure 5.4 Possible scenarios of water transport and storage of South- central Kentucky

The most likely case is a combination of the above scenarios dictated by seasonality, storage, antecedent moisture conditions, and the intensity and amount of rainfall. Further study is needed to determine thresholds and situations where each one is the primary factor for storage and transport. Most importantly, there is a direct and rapid infiltration of water through the epikarst during storm events, which transports contaminants from organic amendments applied to the surface. The karst terrain does not provide a filter for these contaminants, and the rapid input is able to occur throughout the year, even long after the initial application and subsequent rain events have occurred. If

extrapolated to the scale of the agricultural area surrounding the cave in northern Warren County, this would mean an almost ubiquitous amount of fecal coliform pervading the groundwater throughout the year.

5.5 Best Management Practices Recommendations

Animal waste is often applied to row crops as opposed to liquid fertilizers because it is an inexpensive way to add nutrients to the soil and to dispose of the large volumes of manure often accumulated from agricultural practices. There is often a misunderstanding or lack of knowledge about the fate of these contaminants. The BMPs listed in the AWQA were enacted to help maximize crop yields while minimizing ground and surface water contamination. Based upon the findings of this research, testing subsoil and soil water for nutrient rates is as important as testing the upper soils. Within the observed time of application of manure in late December, and the planting of the corn row crop in early April, no crop cover was utilized. During this time contaminants in the manure can easily work their way down to the subsoil. Contaminants such as *E. coli* and FC may diminish from exposure to UV rays in the upper soil, yet still thrive in the subsoil. Deep soil sampling will yield a better understanding of bacteria loading and other nutrient counts.

The utilization of cover crops to maximize nutrient uptake and prevent groundwater contamination and/or leaching into the epikarst is a practice not observed in the study area around Crumps Cave, yet this practice is likely vital for minimizing the accumulation of pollutants in soils, since data collected as part of this research study

indicate animal waste byproducts can remain in the soil-epikarst zone for months after initial application if not absorbed by plant life.

Current BMPs call for no application of organic soil amendments 12 hours prior to a forecasted storm event or 48 hours after a storm event. This study shows direct infiltration of meteoric water associated with significant storm events. This, in return, influences the transport of FC and nutrients through the soil-epikarst system. In karst lands, where water travels to the local aquifer with little physical filtration, timing of application to minimize leaching is of great concern.

Data suggest one of the most influential BMPs for groundwater quality may be the application of fertilizer products during winter months of December- April (Van Donsel *et al.* 1967; Reddy *et al.* 1981). The BMP for winter application suggests avoiding spreading animal waste on frozen or snow-covered land unless conditions allow no other reasonable alternatives and special provisions are made to control runoff and pollution. FC have better survival rates in colder conditions, thus applying animal waste in winter months increases the survivability of FC and allows for their movement into the subsoil, especially when storm events occur just prior to or after application. During this study, data collected supports this BMP. Observed application of organic amendments in the study area was in late December 2010, few weeks prior to the start of data collection. FC and *E. coli* counts from storm events early before growing as well as late in the growing season see high counts of FC and *E. coli*. This study suggests the BMP of not applying until crops have already sprouted to maximize the use of nutrients. This will allow for minimizing loss of nutrients in the root zone and leeching into the subsoil and epikarst zone of the system.

5.6 Importance of Seasonal Storm Event Sampling

Using natural conditions can be more difficult than conducting simulated storm events used in more controlled and laboratory studies. With the difficulties of predicting storm events, best judgment was used for the times when dye was injected into the soil that may have met the threshold. However, the results of this study emphasize the importance of using individual storm event sampling for contaminant transport in karst lands. Seasonal variations in temperature and precipitation create different scenarios for storage and movement of meteoric waters and contaminants through the soil-epikarst system. The four-hour sampling schedule shows the breakthrough for the FC and dye moving through the system and indicated storage and transport better than daily, weekly, or monthly sampling. The methodology presented in this study can apply to tracking the storage and transport of many agriculture contaminants such as nutrients, fertilizer, herbicides and pesticides.

5.7 Conclusions

From the research presented, there are a few conclusions this study provides. The results show that most of the FC and *E. coli* are likely stored in the soil. The dye traces also support this conclusion. If these were primarily stored in the epikarst, we would likely see high counts of them at WF1 all year and not just during storm events. It is this infiltration of meteoric waters exhibited during every season that pushes these bacterial contaminants from their primary soil storage and into the epikarst.

This immense amount of water entering the soil-epikarst system allows for the conditions of not only survival, but the thriving, of FC and *E. coli* in the system. From the

principal factors that determine survivability, the application time during winter and the moisture conditions in the soil gave the bacteria greater survivability due to the cooler temperatures and less precipitation creating runoff.

From the seasonal data, there are two conditions that occur in the soil that dictate the transport of FC and *E. coli*. First, there is a threshold for rain intensity and rain amount that push the bacteria and dye through the soil-epikarst system. Additionally, diffuse flow through conduits adds to the movement of bacteria through the soil- epikarst system. Significant storm events infiltrate the soils and create a high hydraulic head that rapidly pushes the bacteria through main conduits of the epikarst (WF1). This causes a quick drop in SpC and a rise in discharge simultaneously. After the head is lowered, discharge decreases, SpC will slowly rise back toward pre-storm levels and FC counts will decrease. Often, the SpC does not return to previous base flow levels, likely due to the dilution of storage water by rainfall. However, during periods of higher storage, it appears as though continuing recharge and hydraulic pressure pushes out additional storage waters after storm event recovery, and there is a rise in SpC during the falling limb of the discharge curve. During time in-between storms, waters percolate through diffuse conduits as evident by the lower FC and *E. coli* counts and steady rise of SpC.

5.8 Future Studies

The indication of FC and *E. coli* discharging at WF1 is only one example of an epikarstic waterfall in one cave in the study area. There are hundreds of other caves within the study area that could be providing similar contributions to the groundwater

system. Finding the catchment area for WF1 is needed to better aid in understanding the soil-epikarst hydrological characteristics of storage and transport of contaminants in a karst agricultural setting. Measuring the amount of manure applied to the surface and quantifying the FC and *E. coli* discharging from WF1 in colonies/second may help in determining the amount of loading of these bacteria from what is being removed from the soil zone.

Further study needs to be done in the study area on the effects of annual application. Accumulative application of manure will aid the survivability of FC in the soil-epikarst. The data from this study suggest that monitoring and sampling from soil water below the root zone will aid in characterizing of the storage of nutrients in soil. This type of monitoring will also add to understanding transport of bacteria and nutrients as it moves through the soil-epikarst. If BMPs are enacted correctly in a karst landscape the amount of contaminants entering groundwater would be significant lower.

APPENDIX A

sulphorhodamine B at Waterfall One

Julian Decimal Date	Sulphorhodamine B (ppb)
------------------------	----------------------------

56	0.808
----	-------

56.3333	0.173
---------	-------

59.3333	0.547
---------	-------

59.6667	0.115
---------	-------

APPENDIX B

fluorescein at Waterfall One

Julian Decimal Date	Fluorescein (ppb)
55.6667	4.083
56	8.511
56.3333	2.316
56.6667	1.688
57	1.685
57.3333	2.02
57.6667	1.331
58	1.546
58.3333	1.154
58.6667	1.004
59	1.041
59.3333	5.609
59.6667	2.632
60	1.763
60.3333	1.456

60.6667	1.447
61	1.279
61.3333	1.109
61.6667	0.712
62	0.552
63	0.33
63.3333	0.207
63.6667	0.285
64	0.142
66.6667	0.601
67	0.617

APPENDIX C

eosine at Waterfall One

Julian Date	eosine (ppb)
-------------	--------------

116.66	10.153
116.826	9.581
116.993	9.558
117.16	8.586
117.326	131.4
117.493	42.031
117.66	31.429
117.826	31.846
117.993	28.125
118.16	24.932
118.326	23.43

118.493	21.84
118.66	20.165
118.826	19.907
118.993	19.512
119.16	18.737
119.326	18.07
122.493	8.266
122.66	9.349
122.826	12.666
122.993	28.086
123.16	1409.6
123.326	82.6
123.493	25.855
123.66	19.598
123.826	15.767
123.993	17.168
124.16	16.946
124.326	16.237
124.493	16.885
124.66	16.842
124.826	16.793
124.993	16.51
125.16	16.179
125.326	15.853

125.493	15.319
125.993	14.164
126.993	10.615
127.16	10.908
127.326	11.163
127.493	11.186
127.66	10.665
127.826	10.232
127.993	9.84
128.16	10.336
128.326	10.242
128.493	9.871
128.66	9.571
128.826	9.386
128.993	8.832
129.16	8.963
129.326	8.675
129.493	8.58
129.66	8.598
129.826	8.333
129.993	8.008
130.16	7.936
130.326	7.779
130.493	7.562

130.826	7.373
131.16	7.02
131.486	6.774

APPENDIX D

E. coli and fecal coliform at Waterfall One

Julian Decimal Date	<i>E.coli</i> (colonies/100 mL)	Fecal coliform (colonies/100 mL)
18.528	16.1	44.8
19.500	9.7	9.7
20.500	28.2	28.2
24.833	29.9	29.9
25.500	5.2	6.3
25.833	35.5	53.7
26.000	9.8	13.4
26.167	4.1	7.5
26.333	--	3
26.500	1	1
26.667	1	1
26.833	3.1	3.1
27.000	1	1

27.333	4.1	3
32.000	2	2
32.500	9.5	9.5
32.667	727	816.4
32.833	488.4	579.4
33.000	101.7	101.7
33.167	42	50.4
33.333	21.8	26.2
33.667	4.1	4.1
33.833	4.1	5.2
34.000	4.1	6.3
34.167	1	2
34.333	5.2	5.2
34.500	6.3	7.4
34.667	--	1
34.833	2	2
35.000	3.1	3.1
35.167	7.5	7.5
35.333		1
35.500	2	4.1
40.500	1	1
43.486	1	1
54.500	6.3	9.8
61.500	6.3	6.3

75.500	3.1	2
89.500	7.5	7.5
96.451	613	2420
102.451	24196	24196
110.465	1299.7	1732.9
115.500	113.2	174.4
115.667	1089.3	1089.3
115.833	323.2	361.7
116.000	430.3	456.2
116.167	191.8	276.6
116.333	720.9	763
116.500	316.8	608.3
116.667	913	1164
116.833	899	944
117.000	542	716
117.167	1041	1140
117.333	9678	9678
117.500	5199	7945
117.667	4480	5199
117.833	3266	3266
118.000	992	1102
118.167	826	953
118.333	639	689
118.500	606	626

118.667	325	325
118.833	288	335
119.000	205	228
119.167	216	228
119.333	207	240
122.500	142	166
122.667	110	117
122.833	1102	1642
123.000	1376	1828
123.167	7945	7945
123.333	4813	4813
123.667	4480	5199
123.833	944	944
124.000	2595	2595
124.167	1041	1379
124.333	875	1102
124.500	162	205
124.667	245	293
124.833	147	215
125.000	122	160
125.167	110	117
125.333	144	160
248.667	--	9208
249.000	--	4106

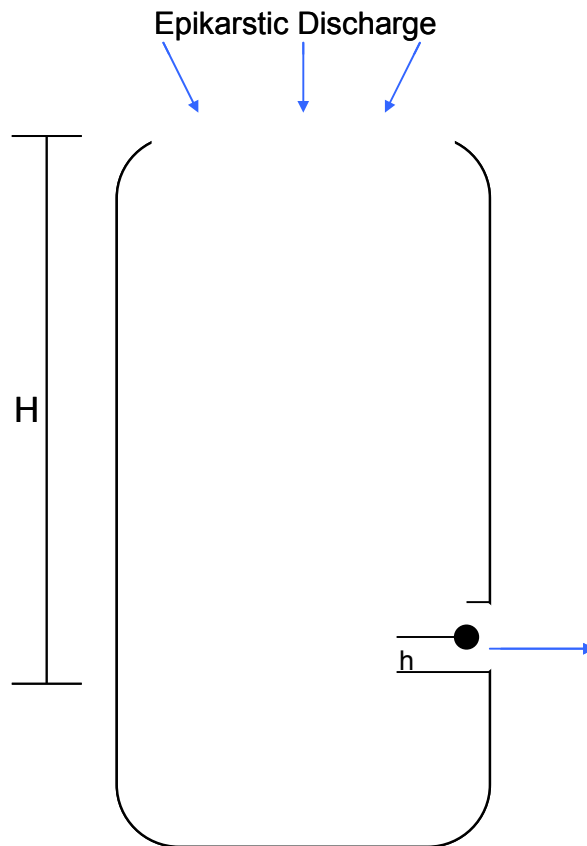
249.333	--	455
249.500	--	384
249.833	--	393
250.167	--	279
250.333	--	265
250.500	--	313
250.667	--	231
250.833	--	256
251.333	--	355

APPENDIX E

Equation for discharge barrel

$$Q = C_D \int_0^{\min(2R, h)} \sqrt{2y(h-y)(R^2 - (R-y)^2)} dy$$

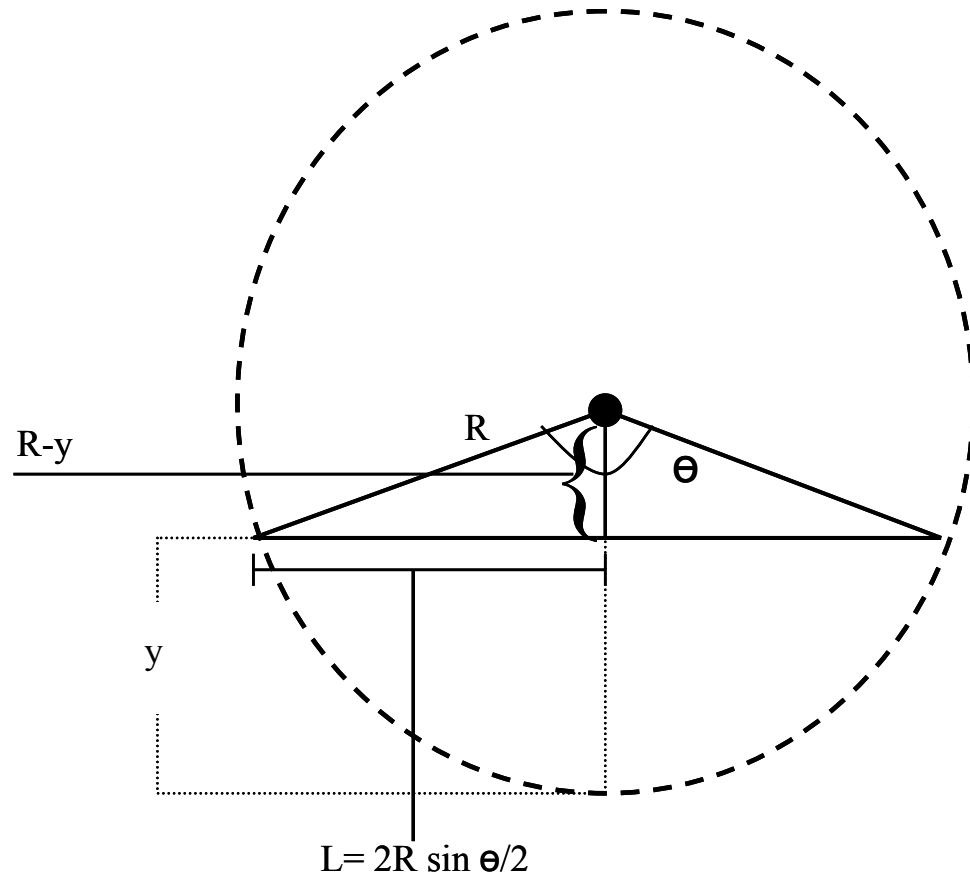
Parameters for equation



H= height to bottom of hole from top of barrel

h= height from bottom of hole to middle of hole

Aperture of discharge hole



R = radius
 y = elevation above bottom of hole
 θ = theta

REFERENCES

- Bakalowicz, M. 2003. Epikarst, the skin of karst. Conference on Epikarst, The Karst Waters Institute, Shepherdstown, West Virginia
- Birk, S., Geyer, T., Liedl, R., Sauter, M. 2005. Process-based interpretation of tracer tests in carbonate aquifers. *GroundWater*. 43 (3): 381–388.
- Bohlke, J. 2002. Groundwater recharge and agricultural contamination. *Hydrogeology Journal*. 10: 53-179.
- Bolster, C., Grover, C., Meiman, J., Fernandez-Cortez, A., Crockett, C. 2006. Practical limits of high-resolution evaluation of carbonate chemistry within karst flow systems. Proceedings of the Conference on Limestone Hydrogeology.
- Brahana, J., Hays, P., Kresse, T., Sauer, T., Stanton, G. 1999. The Savoy Experimental Watershed - early lessons for hydrogeologic modeling from a well-characterized karst research site. In A.N. Palmer et al. (ed.) Karst modeling, Karst Waters Institute Special Publication. 5: 247-254.
- Center for disease control. Outbreak of West Nile-Like Viral Encephalitis. New York, October 1st, 1999.
<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4838a1.htm>.
Accessed [11/23/2011]
- Chandler, D. and Craven, J. 1980. Relationship of soil moisture to survival of *Escherichia coli* and *Salmonella typhimurium* in soils. *Australian Journal of Agricultural Research*. 31: 547-555.
- Crane, S.R., Westerman, P and Overcash, M. 1981. Dieoff of fecal indicator organisms following land application of poultry manure. *Journal of Environmental Quality*. 9: 531-537.
- Crane, S. and Moore, J. 1986. Modeling enteric bacterial die-off: A review. *Water Air Soil Pollution*. 27: 411–439.
- Currens, J. 2002. Kentucky is Karst Country: What you should know about sinkholes and springs. Kentucky Geological Survey. Information Circular 004 Series 12
- Drever, J. 1988. *The Geochemistry of Natural Waters*. Prentice Hall, Inc. New Jersey.
- Drew, D. and Holtz, H. 1999. *Karst Hydrogeology and Human Activities. Impacts, Consequences and Implications*. Rotterdam, Brookfield. Balkema.

- Ellis, J. and McCalla, T. 1976. Fate of pathogens in soils receiving animal wastes – A review. ASAE Paper No. 762560. St. Joseph, MI: ASAE.
- Filip, Z., Kaddu-Mulindwa, D., and Milde, G. 1988. Survival of some pathogenic and facultative pathogenic bacteria in groundwater. *Water Science and Technology* 20: 227-231.
- Ford, D.C. and Williams, P.W. 2007. *Karst Hydrology and Geomorphology*. West Sussex, England: John Wiley and Sons Ltd.
- Friederich H. and Smart P. 1981. Dye tracer studies of the unsaturated-zone recharge of the Carboniferous Limestone aquifer of the Mendip Hills, England. Proceedings of the 8th International Congress of Speleology. 1: 283–6.
- Gagliardi, J.V. and J.S. Karns. 2000. Leaching of *Escherichia coli* in diverse soils under various agricultural management practices. *Applied and Environmental Microbiology*. 66:877-883.
- Gerba, C., Wallis, C., Melnick, J. 1975. Fate of wastewater bacteria and viruses in soil. *Journal of Irrigation and Drainage Engineering*. 101: 157-174.
- Göppert, N. and Goldscheider, N. 2007. Solute and colloid transport in karst conduits under low and high flow conditions. *Groundwater*.
<http://info.ngwa.org/gwol/pdf/081882558.pdf>.
 Accessed [11/23/2011]
- Grasso, A., Jeannin, P., Zwahlen, F. 2003. A deterministic approach to the coupled analysis of karst spring hydrographs and chemographs. *Journal of Hydrology*. 271: 65–76.
- Groves, C. and Meiman, J. 2005. Weathering, geomorphic work, and karst landscape evolution in the Cave City groundwater basin, Mammoth Cave, Kentucky. *Geomorphology*. 67: 115-126.
- Groves, C., Bolster, C., Meiman, J. 2005. Spatial and temporal variations in epikarst storage and flow in south central Kentucky's Pennyroyal Plateau sinkhole plain. Proceedings of the USGS Karst Interest Group Conference.
- Hagedorn, C., Hansen, D., and Simonson, G. 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. *Journal of Environmental Quality*. 7: 55-59.

- Ham, B., Fowler, R., Groves, C., Bolster, C. 2009. Using conservation and biological tracers to better understand the transport of agricultural contaminants from soil water through the epikarst zone. *Proceedings of the International Congress of Speleology*. 3: 1519-1523.
- Hess, J. 1974. *Hydrochemical Investigations of the central Kentucky Karst Aquifer System*. Ph.D. thesis, Department of Geosciences, The Pennsylvania State University.
- Hess, J., Wells, S., Quinlan, J., and White, W. 1989. Hydrogeology of the south-central Kentucky karst. In *Karst Hydrology Concepts from the Mammoth Cave Area*, William B. White and Elizabeth L. White, eds., Van Nostrand Reinhold, New York.
- Hess, J.W. and White, W.B. 1988. Storm response of the karstic carbonate aquifer of southcentral Kentucky. *Journal of Hydrology*. 99: 235-252.
- Hess, J.W. and White W.B., 1989. Water budget and physical hydrology. In *Karst Hydrology Concepts from the Mammoth Cave Area*, William B. White and Elizabeth L. White, eds. Van Nostrand Reinhold, New York.
- Howell, J.M., M.S. Coyne and P.L. Cornelius. 1995. Fecal bacteria in agricultural waters of the bluegrass region of Kentucky. *Journal of Environmental Quality*. 24: 411-419.
- Jamieson, R., Gordon, R., Sharples, K., Stratton, G., Madani, A. 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Canadian Biosystems Engineering*. 44: 1.1–1.9.
- Jones, W.K., 1997, Karst hydrology atlas of West Virginia. Karst Waters Institute, Charles Town, W.Va. pp.111
- Kaufmann, G. and Dreybrodt, W. 2007. Calcite dissolution kinetics in the system $\text{CaCO}_3\text{-H}_2\text{O-CO}_2$ at high undersaturation. *Geochimica et Cosmochimica Acta*. 71: 1398-1410.
- Klimchouk, A.B. 2000. The formation of epikarst and its role in vadose speleogenesis. In: *Speleogenesis: Evolution of karst aquifers*. Huntsville: National. Speleological . Society. 91-99.
- Klimchouk A (2004) Towards defining, delimiting and classifying epikarst: Its origin, processes and variants of geomorphic evolution. *Speleogenesis* 2: 1–13

- Lee, E., and Krothe, N., 2001. A four component mixing model for water in a karst terrain in south-central Indiana, USA. Using solute concentration and stable isotope as tracers. *Chemical Geology*. 179: 129–143.
- Mahler, B.J., Personne, J.C., Lods, G.F., Drogue, C. 2000. Transport of free and particulate-associated bacteria in karst. *Journal of Hydrology*. 238: 179–193
- Mitchell, A., Bramley, R., and Johnson, A. 2005. Export of nutrients and suspended sediment during a cyclone-mediated flood event in the Herbert River catchment, Australia. *Marine and Freshwater Research*. 48(1): 79 - 88.
- Mubiru, D.N., M.S. Coyne and J.H. Grove. 2000. Mortality of *Escherichia coli* O157:H7 in two soils with different physical and chemical properties. *Journal of Environmental Quality*. 29: 1821-1825.
- Nicholson, F., Hutchison, M., Smith, K., Keevil, C., Chambers, B., and Moore, A. 2000. A study on farm manure application to agricultural land and an assessment of the risks of pathogen transfer into the food chain project number Fs2566, London: Final Report to the Ministry of Agriculture, Fisheries and Food.
- Nguyet, V., Groves, C., Bolster, C.H. 2010. Seasonal Variations in Background Hydrochemistry of Epikarst Waters in Kentucky's Pennyroyal Plateau. National Speleological Society Annual Meeting.
- NOAA. National Climatic Data Center
<http://www.ncdc.noaa.gov/oa/ncdc.html>.
 Accessed [11/23/2011]
- Panno, S., Kelly, W., Weibel, C., Krapac, I., and Sargent, S. 2003. Water quality and agrichemical loading in two groundwater basins of Illinois' sinkhole plain. Illinois State Geological Survey Environmental Geology Series 156. Champaign, Illinois.
- Perrin, K., Jeannin, P., Zwahlen, F. 2003. Epikarst storage in a karst aquifer: a conceptual model based on isotopic data, Milandre test site, Switzerland. *Journal of Hydrology*. 279: 106–124
- Quinlan J. and Ewers R. 1985. Ground water flow in limestone terrines: strategy rationale and procedure for reliable, efficient monitoring of ground water quality in karst areas. *5th national symposium and exposition on aquifer restoration and ground water monitoring*. National Water Well Association, Columbus, Ohio. 197–234
- Raeisi, E., Groves, C., Groves, and Meiman, J. 2007. Effects of partial and full pipe flow on hydrochemographs of the Mammoth Cave Karst Aquifer, *Journal of Hydrology*. 337: 1-10.

- Ray, J.A. and Currens, J.C. 1998. Mapped karst groundwater basins in the Beaver Dam 30 x 60 Minute Quadrangle, Kentucky Geological Survey.
- Ray, J.A. and Currens, J.C. 2000. Mapped karst groundwater basins in the Campbellsville 30 x 60 Minute Quadrangle, Kentucky Geological Survey.
- Ray, J.A. and Blair, R.J. 2005. Large perennial springs of Kentucky: Their identification, base flow, catchment, and classification. *in* Beck, B.F. (ed) Sinkholes and the Engineering and Environmental Impact of Karst. Geotechnical Special Publication 144. American Society of Civil Engineers, Reston, Virginia. 410-422.
- Reddy, K., Khaleel, R, and Overcash, M. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. *Journal of Environmental Quality*. 10: 255-266.
- Richards, P.W. 1964. Geologic map of the Smiths Grove quadrangle, Kentucky. US Geological Survey Geologic Quadrangle Map GQ 357.
- Ryan, M., and Meiman, J. 1996. An examination of short-term variations in water quality at a karst spring in Kentucky. *Ground Water*. 34: 23–30.
- Saini, R., Halverson, L., and Lorimor, J. 2003. Rainfall timing and frequency influence on leaching of *Escherichia coli* RS2G through soil following manure application. *Journal of Environmental Quality*. 32: 1865-1872
- Soil Survey Staff NRCS. 2011. United States Department of Agriculture. Web Soil Survey.
<http://websoilsurvey.nrcs.usda.gov/>.
 Accessed [11/7/2011].
- Tate, R. 1978. Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. *Applied and Environmental Microbiology*. 35: 925-929
- Toran, L., Herman, E.K., White, W.B., 2007. Comparison of flowpaths to a well and spring in a karst aquifer. *Ground Water*. 45 (3): 281–287.
- Tyrrel, S., and Quinton, J. 2003. Overland flow transport of pathogens from agricultural land receiving fecal wastes. *Journal of Applied Microbiology*. 94(S1): 87–93.
- United States Geological Survey. 1999. The Quality of Our Nation's Water-Nutrients and Pesticides. U.S. Geological Survey Circular 1225.
- USEPA. 1986. Ambient water quality criteria for bacteria. EPA440/5–84–002. Washington, D.C.: USEPA Office of Water Regulations and Standards.

- Van Donsel, D.J., Geldreich, E. E., Clarke, N.A. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm water pollution *Applied Microbiology*. 15: 1362-1370.
- White, P.W. 2008. The role of the epikarst in karst and cave hydrology; a review. *International Journal of Speleology*. 37 (1): 1-10
- White, William B. 1988. *Geomorphology and Hydrology of Karst Terrains*. Oxford University Press: New York.
- Williams, P.W. 1983. The role of the subcutaneous zone in karst hydrology. *Journal of Hydrology*. 61: 45-67.
- Williams, P.W. 2008. The role of the epikarst in karst and cave hydrogeology: A review. *Journal of Hydrology : a review. International Journal Of Speleology*. 37: 1-10.
- Zhai, Q., Coyne, R., Barnhisel, R.I. 1995. Mortality rates of fecal bacteria in subsoil amended with poultry manure. *Bioresource Technology*. 54: 165-169

